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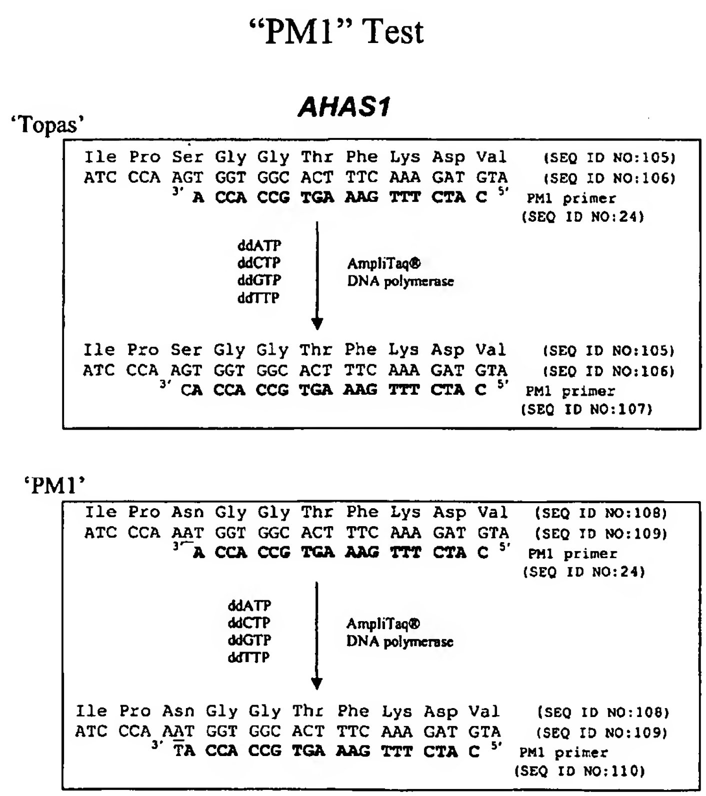
(54) Title: COMPOSITIONS AND METHODS FOR IDENTIFYING PLANTS HAVING INCREASED TOLERANCE TO IMIDAZOLINONE HERBICIDES



(57) Abstract: The present invention provides compositions and methods for assaying commercially relevant imidazolinone herbicide tolerance conferred by a *Brassica napus AHAS1* PM1 mutation and a *Brassica napus AHAS3* PM2 mutation in a plant.

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COMPOSITIONS AND METHODS FOR IDENTIFYING PLANTS HAVING INCREASED TOLERANCE TO IMIDAZOLINONE HERBICIDES

BACKGROUND OF THE INVENTION

Field of the Invention

[001] This invention relates generally to compositions and methods for identifying *Brassica* plants having increased tolerance to an imidazolinone herbicide.

Background Art

[002] Canola is the seed derived from any of the *Brassica* species *B. napus*, *B. campestris/rapa*, and certain varieties of *B. juncea*. Canola oil is high in monounsaturated fats, moderate in polyunsaturated fats, and low in saturated fats, having the lowest level of saturated fat of any vegetable oil. Thus canola oil is an important dietary option for lowering serum cholesterol in humans. In addition, the protein meal which is the byproduct of canola oil production has a high nutritional content and is used in animal feeds.

[003] Imidazolinone and sulfonylurea herbicides are widely used in modern agriculture due to their effectiveness at very low application rates and relative non-toxicity in animals. Both of these herbicides act by inhibiting acetohydroxyacid synthase (AHAS; EC 4.1.3.18, also known as acetolactate synthase or ALS), the first enzyme in the synthetic pathway of the branched chain amino acids valine, leucine and isoleucine. Several examples of commercially available imidazolinone herbicides are PURSUIT® (imazethapyr), SCEPTER® (imazaquin) and ARSENAL® (imazapyr). Examples of sulfonylurea herbicides are chlorsulfuron, metsulfuron methyl, sulfometuron methyl, chlorimuron ethyl, thifensulfuron methyl, tribenuron methyl, bensulfuron methyl, nicosulfuron, ethametsulfuron methyl, rimsulfuron, triflusulfuron methyl, triasulfuron, primisulfuron methyl, cinosulfuron, amidosulfuron, fluzasulfuron, imazosulfuron, pyrazosulfuron ethyl and halosulfuron.

[004] Due to their high effectiveness and low toxicity, imidazolinone herbicides are favored for application to many crops, including canola, by spraying over the top of a wide area of vegetation. The ability to spray an herbicide over the top of a wide range of vegetation decreases the costs associated with plantation establishment and maintenance and

decreases the need for site preparation prior to use of such chemicals. Spraying over the top of a desired tolerant species also results in the ability to achieve maximum yield potential of the desired species due to the absence of competitive species. However, the ability to use such spray-over techniques is dependent upon the presence of imidazolinone resistant species of the desired vegetation in the spray over area. In addition, because residual imidazolinones persist in a sprayed field, a variety of resistant species is advantageous for crop rotation purposes.

[005] Unfortunately, the *Brassica* species which are the source of canola are closely related to a number of broad leaf cruciferous weeds, for example, stinkweed, ball mustard, wormseed mustard, hare's ear mustard, shepherd's purse, common peppergrass, flixweed, and the like. Thus it was necessary to develop *Brassica* cultivars which are tolerant or resistant to the imidazolinone herbicides. Swanson, *et al.* (1989) *Theor. Appl. Genet.* **78**, 525-530 discloses *B. napus* mutants P_1 and P_2, developed by mutagenesis of microspores of *B. napus* (cv 'Topas'), which demonstrated tolerance to the imidazolinone herbicides PURSUIT® and ASSERT® at levels approaching ten times the field-recommended rates. The homozygous P_2 mutant produced an AHAS enzyme which was 500 times more tolerant to PURSUIT® than wild type enzyme, while the AHAS enzyme from the homozygous P_1 mutant was only slightly more tolerant than the wild type enzyme. In field trials, the P_1, P_2, and P_1 x P_2 hybrid withstood ASSERT® applications up to 800 g/ha with no loss of yield. The P_1 and P_2 mutations were unlinked and semidominant, and P_1 x P_2 crosses tolerated levels of PURSUIT® higher than those tolerated by either homozygous mutant. Imidazolinone-tolerant cultivars of *B. napus* were developed from the P_1 x P_2 mutants and have been sold as CLEARFIELD® canola. *See also*, Canadian patent application number 2,340,282; Canadian patent number 1,335,412, and European patent number 284419.

[006] Rutledge, *et al.* (1991) *Mol. Gen. Genet.* **229**, 31-40) discloses the nucleic acid sequence of three of the five genes encoding AHAS isoenzymes in *B. napus*, *AHAS1*, *AHAS2*, and *AHAS3*. Rutledge, *et al.* discusses the mutants of Swanson, *et al.* and predicts that the two alleles that conferred resistance to imidazolinone herbicides correspond to *AHAS1* and *AHAS3*. Hattori *et al.* (1995) *Mol. Gen. Genet.* **246**, 419-425 disclose a mutant allele of *AHAS3* from a mutant *B. napus* cv Topas cell suspension culture line in which a single nucleotide change at codon 557 leading to an amino acid change from tryptophan to leucine confers resistance to sulfonylurea, imidazolinone, and triazolopyrimidine herbicides. Codon 557 of Hattori, *et al.* corresponds to codon 556 of the *AHAS3* sequence disclosed in

Rutledge, *et al., supra*, and to codon 556 of the *AHAS3* sequence set forth as GENBANK accession number gi/17775/emb/Z11526/.

[007] A single nucleotide mutation at codon 173 in a *B. napus* ALS gene, corresponding to *AHAS2* of Rutledge *et al., supra*, leads to a change from Pro to Ser (Wiersma *et al.* (1989) *Mol. Gen. Genet.* **219**, 413-420). The mutant *B. napus AHAS2* gene was transformed into tobacco to produce a chlorsulfuron tolerant phenotype.

[008] U.S.Pat.Nos. 6,114,116 and 6,358,686 disclose nucleic acid sequences from *B. napus* and *B. oleracea* containing polymorphisms, none of which appears to correspond to the polymorphism disclosed in Hattori, *et al., supra.*

[009] For commercially relevant *Brassica* cultivars, it is necessary to ensure that each lot of herbicide-resistant seed contains all mutations necessary to confer herbicide tolerance. A method is needed to detect mutations in *Brassica AHAS1* and *AHAS3* genes that confer increased imidazolinone tolerance to commercial cultivars.

SUMMARY OF THE INVENTION

[010] The present invention describes the location and identity of a single nucleotide polymorphism at position 1937 of the *AHAS1* gene of *B. napus*, the polymorphism being designated as the PM1 mutation. The PM1 mutation confers about 15% of the tolerance to imidazolinone herbicides that is present in CLEARFIELD® canola. CLEARFIELD® canola also contains a second single nucleotide polymorphism at position 1709 of the *AHAS3* gene of *B. napus*, which corresponds to the tryptophan to leucine substitution described in Hattori *et al., supra*. For the purpose of the present invention, this polymorphism is designated as the PM2 mutation. The PM2 mutation confers about 85% of the tolerance to imidazolinone herbicides exhibited by CLEARFIELD® canola. Both the PM1 and PM2 mutations are required to produce a *Brassica* plant with sufficient herbicide tolerance to be commercially relevant, as in CLEARFIELD® canola.

[011] Accordingly, the present invention provides methods of identifying a plant having increased tolerance to an imidazolinone herbicide by detecting the presence or absence of the *B. napus* PM1 and PM2 mutations in the plant. One of the advantages of the present invention is that it provides a reliable and quick means to detect plants with commercially relevant imidazolinone tolerance.

[012] In one embodiment, the invention provides a method of assaying a plant for imidazolinone herbicide resistance conferred by the combination of the PM1 mutation of the *B. napus AHAS1* gene and the PM2 mutation of the *B. napus AHAS3* gene. In this method,

genomic DNA is isolated from the plant, the presence or absence of the PM1 mutation is determined, and the presence or absence of the PM2 mutation is determined, wherein the presence of the PM1 mutation and the PM2 mutation is indicative of commercially relevant imidazolinone tolerance in the plant.

[013] In another embodiment, the invention provides novel polynucleotide primers useful for detecting the PM1 and PM2 mutations.

BRIEF DESCRIPTION OF THE DRAWINGS

[014] Figure 1A shows the nucleic acid and amino acid sequences of *B. napus AHAS1* containing the PM1 mutation (SEQ ID NO:1 and SEQ ID NO:101, respectively).

[015] Figure 1B shows the nucleic acid and amino acid sequences of *B. napus AHAS3* containing the PM2 mutation (SEQ ID NO:2 and SEQ ID NO:102, respectively).

[016] Figure 1C shows the nucleic acid and amino acid sequences of wild type *B. napus* cv. 'Topas' *AHAS1* (SEQ ID NO:3 and SEQ ID NO:103, respectively).

[017] Figure 1D shows the nucleic acid and amino acid sequences of wild type *B. napus AHAS3* Topas cv. (SEQ ID NO:4 and SEQ ID NO:104, respectively).

[018] Figure 1E is a table setting forth the sequences of various oligonucleotides (SEQ ID NOs: 5-88) useful in determining the presence or absence of the PM1 and PM2 mutations in accordance with the invention.

[019] Figure 2 is a schematic representation of one embodiment of the PM1 mutation determination step of a primer extension-based assay of the invention. The coding strand is shown with the amino acid translation of the codons. The wild type plant is denoted as 'Topas' (SEQ ID NOs: 105, 106, 24, 105, 106, and 107, respectively, in order of appearance) and the mutated plant is denoted as 'PM1' (SEQ ID NOs: 108, 109. 24, 108, 109, and 110, respectively, in order of appearance). The mutated nucleotide "A" is underlined on the coding strand. The PM1 extension primer is indicated in bold and is placed at its annealing site on *AHAS1*.

[020] Figure 3 is a schematic representation of one embodiment of the PM2 mutation determination step of a primer extension-based assay of the invention. The coding strand is shown with the amino acid translation of the codons. The wild type plant is denoted as 'Topas' (Seq ID NOs: 111, 112, 66, 111, 112, and 113, respectively, in order of appearance) and the mutated plant is denoted as 'PM2' (SEQ ID NOs: 114, 115, 66, 114, 115, and 116, respectively, in order of appearance). The mutated nucleotide "T" is

underlined on the coding strand. The PM2 extension primer is indicated in bold and is placed at its annealing site on *AHAS3*.

[021] Figure 4 is a table describing the predicted phenotypes of double haploid *B. napus* plants used to validate the method of the invention.

[022] Figure 5 is a table describing the results of the method of the invention in an embodiment employing the ABI PRISM® SNP detection system.

[023] Figure 6 is a table describing the results of the method of the invention in an embodiment employing the PYROSEQUENCING™ detection system.

DETAILED DESCRIPTION OF THE INVENTION

[024] The present invention provides methods and compositions for identifying plants having increased tolerance to an imidazolinone herbicide by virtue of the presence of the *B. napus* PM1 and PM2 mutations. More particularly, the methods and compositions of the present invention allow identification of *Brassica* seeds and plants having commercially relevant imidazolinone tolerance, such as CLEARFIELD® canola. In some embodiments, the methods of the invention employ novel polynucleotide primers including PM1 extension primers and PM2 extension primers.

[025] It is to be understood that as used in the specification and in the claims, "a" or "an" can mean one or more, depending upon the context in which it is used. Thus, for example, reference to "a cell" can mean that at least one cell can be utilized.

[026] For the purposes of the present invention, the level of tolerance to imidazolinone herbicides exhibited by CLEARFIELD® canola which contains both the PM1 and PM2 mutations is defined as 100% tolerance, or "commercially relevant imidazolinone tolerance" or "commercial field tolerance". The terms "tolerance" and "resistance" are used interchangeably herein.

[027] "Homologs" are defined herein as two nucleic acids or polypeptides that have similar, or "identical", nucleotide or amino acid sequences, respectively. Homologs include allelic variants, analogs, orthologs and paralogs. As used herein, the term "allelic variant" refers to a nucleotide sequence containing polymorphisms that lead to changes in the amino acid sequences of AHAS proteins and that exist within a natural population (e.g., a plant species or variety). As used herein, the term "analogs" refers to two nucleic acids that have the same or similar function, but that have evolved separately in unrelated organisms. The term "orthologs" refers to two nucleic acids from different species, but that have evolved from a common ancestral gene by speciation. Normally, orthologs encode polypeptides

having the same or similar functions. As also used herein, the term "paralogs" refers to two nucleic acids that are related by duplication within a genome. Paralogs usually have different functions, but these functions may be related (Tatusov, R.L. *et al.*, 1997 Science 278(5338):631-637).

[028] As defined herein, a "PM1 mutation" refers to a single nucleotide polymorphism in a *B. napus AHAS1* gene in which there is a "G" to "A" nucleotide substitution at position 1937 of the *AHAS1* wildtype polynucleotide sequence shown in Figure 1C (SEQ ID NO:3) or at a nucleotide position that corresponds to position 1937 in an *AHAS1* homolog, which substitution leads to a serine to asparagine amino acid substitution at position 638 in the *B. napus* AHAS1 enzyme.

[029] A "PM1 oligonucleotide" refers to an oligonucleotide sequence corresponding to a PM1 mutation. An oligonucleotide as defined herein is a nucleic acid comprising from about 8 to about 25 covalently linked nucleotides. In accordance with the invention, an oligonucleotide may comprise any nucleic acid, including, without limitation, phosphorothioates, phosphoramidates, peptide nucleic acids, and the like. As defined herein, "corresponding to a PM1 mutation" includes the following: an oligonucleotide capable of specific hybridization to a region of an *AHAS1* gene which is 5′ of position 1937 of the *AHAS1* gene as set forth in SEQ ID NO:3 (for example, an oligonucleotide comprising any one of SEQ ID NO:5; SEQ ID NO:6; SEQ ID NO:7; SEQ ID NO:8; SEQ ID NO:9; SEQ ID NO:10; SEQ ID NO:11; SEQ ID NO:12; SEQ ID NO: 13; SEQ ID NO:14; SEQ ID NO:15; SEQ ID NO:16; SEQ ID NO:17; SEQ ID NO:18; SEQ ID NO: 19; SEQ ID NO:20; SEQ ID NO:21; SEQ ID NO:22; or SEQ ID NO:23 as set forth in Figure 1E); an oligonucleotide capable of specific hybridization to a region of an *AHAS1* gene which is 3′ of position 1937 of the *AHAS1* gene as set forth in SEQ ID NO:3 (for example, an oligonucleotide comprising any one of SEQ ID NO:24; SEQ ID NO:25; SEQ ID NO:26; SEQ ID NO:27; SEQ ID NO: 28; SEQ ID NO:29; SEQ ID No;30; SEQ ID NO:31; SEQ ID NO:32; SEQ ID NO:33; SEQ ID NO:34; SEQ ID NO:35; SEQ ID NO:36; SEQ ID NO:37; SEQ ID NO:38; SEQ ID NO:39; SEQ ID NO:40; SEQ ID NO:41; or SEQ ID NO:42 as set forth in Figure 1E); an oligonucleotide capable of specific hybridization to a region of the *AHAS1* gene which spans position 1937 of the *AHAS1* gene as set forth in SEQ ID NO:3 (for example, an oligonucleotide comprising SEQ ID NO: 45 as set forth in Figure 1E); an oligonucleotide capable of specific hybridization to a region of an *AHAS1* gene which is 5′ of position 1937 of the complement of the *AHAS1* gene set forth in SEQ ID NO:3; an oligonucleotide capable of specific hybridization to a region of an *AHAS1* gene which is 3′ of position 1937 of the

complement of the *AHAS1* gene set forth in SEQ ID NO:3; and an oligonucleotide capable of specific hybridization to a region of the *AHAS1* gene which spans position 1937 of the complement of the *AHAS1* gene as set forth in SEQ ID NO:3 (for example, an oligonucleotide comprising SEQ ID NO: 46 as set forth in Figure 1E). The term "nucleic acid" includes RNA or DNA that is linear or branched, single or double stranded, or a hybrid thereof. These terms also encompass RNA/DNA hybrids.

[030] As defined herein, a "PM2 mutation" refers to a single nucleotide polymorphism in a *B. napus AHAS3* gene in which there is a "G" to "T" nucleotide substitution at position 1709 of the *AHAS3* wildtype polynucleotide sequence shown in Figure 1D (SEQ ID NO:4) or at a nucleotide position that corresponds to position 1709 in an *AHAS3* homolog, which substitution leads to a tryptophan to leucine amino acid substitution at position 556 in the *B. napus* AHAS3 enzyme.

[031] A "PM2 oligonucleotide" refers to an oligonucleotide sequence corresponding to a PM2 mutation. As defined herein, "corresponding to a PM2 mutation" includes the following: an oligonucleotide capable of specific hybridization to a region of an *AHAS3* gene which is 5′ of position 1709 of the *AHAS3* gene as set forth in SEQ ID NO:4 (for example, an oligonucleotide comprising any one of SEQ ID NO:47; SEQ ID NO:48; SEQ ID NO:49; SEQ ID NO:50; SEQ ID NO:51; SEQ ID NO:52; SEQ ID NO:53; SEQ ID NO:54; SEQ ID NO:55; SEQ ID NO:56; SEQ ID NO:57; SEQ ID NO:58; SEQ ID NO:59; SEQ ID NO:60; SEQ ID NO:61; SEQ ID NO:62; SEQ ID NO:63; SEQ ID NO:64; or SEQ ID NO:65 as set forth in Figure 1E); an oligonucleotide capable of specific hybridization to a region of an *AHAS3* gene which is 3′ of position 1709 of the *AHAS3* gene as set forth in SEQ ID NO:4 (for example, an oligonucleotide comprising any one of SEQ ID NO:66; SEQ ID NO:67; SEQ ID NO:68; SEQ ID NO:69; SEQ ID NO:70; SEQ ID NO:71; SEQ ID NO:72; SEQ ID NO:73; SEQ ID NO:74; SEQ ID NO:75; SEQ ID NO:76; SEQ ID NO:77; SEQ ID NO:78; SEQ ID NO:79; SEQ ID NO:80; SEQ ID NO:81; SEQ ID NO:82; SEQ ID NO:83; and SEQ ID NO:84 as set forth in Figure 1E); an oligonucleotide capable of specific hybridization to a region of the *AHAS3* gene which spans position 1709 of the *AHAS3* gene as set forth in SEQ ID NO:4 (for example, an oligonucleotide comprising SEQ ID NO: 85 as set forth in Figure 1E); an oligonucleotide capable of specific hybridization to a region of an *AHAS3* gene which is 5′ of position 1709 of the complement of the *AHAS3* gene set forth in SEQ ID NO:4; an oligonucleotide capable of specific hybridization to a region of an *AHAS3* gene which is 3′ of position 1709 of the complement of the *AHAS3* gene set forth in SEQ ID NO:4; and an oligonucleotide capable of specific hybridization to a region of the *AHAS3*

gene which spans position 1709 of the complement of the *AHAS3* gene as set forth in SEQ ID NO:4 (for example, an oligonucleotide comprising SEQ ID NO: 86 as set forth in Figure 1E).

[032] Also encompassed in the present invention are oligonucleotides corresponding to the wild type alleles at the PM1 and PM2 mutations which are useful as controls in the SNP detection assays. For example, an oligonucleotide corresponding to position 1937 of the *AHAS1* gene set forth in SEQ ID NO:1, comprising a sequence selected from the group consisting of SEQ ID NO:43 and SEQ ID NO:44 as set forth in Figure 1E, is useful as a control in a SNP assay for the PM1 mutation. Similarly, an oligonucleotide corresponding to position 1709 of the *AHAS3* gene set forth in SEQ ID NO:2, comprising a sequence selected from the group consisting of SEQ ID NO:85 and SEQ ID NO:86 as set forth in Figure 1E, is useful as a control in a SNP assay for the PM2 mutation.

[033] The presence of the PM1 and PM2 mutations in a plant may confer tolerance to such imidazolinone herbicides as PURSUIT® (imazethapyr, 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid), CADRE® (imazapic, 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-methyl-3-pyridinecarboxylic acid), RAPTOR® (imazamox, 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-(methoxymethyl)-3-pyridinecarboxylic acid), SCEPTER® (imazaquin, 2-(4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl)-3-quinolinecarboxylic acid), ASSERT® (imazethabenz, methyl esters of 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-4-methylbenzoic acid and 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-methylbenzoic acid), ARSENAL® (imazapyr, 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-3-pyridinecarboxylic acid), and the like. In addition, the PM1 and PM2 mutations may confer resistance to sulfonylurea and triazolopyrimidine herbicides.

[034] The PM1 and PM2 mutations may be present in a plant by virtue of mutagenesis of any species of plant containing the *B. napus AHAS1* and *AHAS3* genes, respectively. Alternatively, the PM1 and PM2 mutations may be present in a plant by virtue of transformation of the *B. napus AHAS1* PM1 gene and the *B. napus AHAS3* PM2 genes into the plant, using known methods such as those set forth in U.S.Pat.Nos. 5,591,616; 5,767,368; 5,736,369; 6,020,539; 6,153,813; 5,036,006; 5,120,657; 5,969,213; 6,288,312; 6,258,999, and the like. Preferably, the plant is a *Brassica* oilseed. More preferably, the plant species is selected from the group consisting of *B. napus*, *B. campestris/rapa*, and *B. juncea*. Most

preferably, the plant species is *B. napus*. In accordance with the present invention, the term "plant" includes seeds, leaves, stems, whole plants, organelles, cells, and tissues.

[035] In the first step of the method of the invention, genomic DNA is isolated from the plant. It is to be understood that when practicing the method of the present invention, genomic DNA can be extracted from the plant by any method known to those of skill in the art. Genomic DNA can be extracted from a whole plant, a plant leaf, a plant stem, a plant seed, or any plant organelle, cell or tissue. One non-limiting method for extracting the DNA from a plant leaf is described in Example 1 below.

[036] In the second step of the method of the invention, the presence or absence of the PM1 mutation in the extracted DNA is determined. In the third step of the invention, the presence or absence of the PM2 mutation in the extracted DNA is determined. In accordance with the invention, the steps of detecting the PM1 and PM2 mutations may be performed in any order, or simultaneously.

[037] Any method may be used to detect the PM1 and PM2 mutations. For example, commercially available single nucleotide polymorphism (SNP) detection systems may be used, such as the SNP-IT™ system (Orchid Biosciences, Princeton, NJ), the MassArray™ System (Sequenom, Inc., San Diego, CA), the BeadArray™ System (Illumina, San Diego, CA), the ABIPrism Genetic Analyzer (Applied Biosystems, Foster City, CA), the ALFexpress™ (Amersham Biosciences, Buckinghamshire, UK), the PSQ™96 System (Pyrosequencing AB, Uppsala, Sweden), the Invader™ assay (Third Wave Agbio, Inc., Madison, WI), and the like. A variety of methods exist for identification of a nucleotide at a polymorphic site in a nucleic acid, as described, for example, in U.S.Pat.Nos. 6,087,095; 6,046,005; 6,017,702; 5,981,186; 5,976,802; 5,928,906; 5,912,118; 5,908,755; 5,869,242; 5,853,979; 5,849,542; 5,834,189; 4,851,331; 4,656,127; 5,679,524; 6,004,744; 6,013,431; 6,210,891; 6,183,958; 5,958,692; 5,851,770; 6,110,684; 5,856,092; 5,605,798; 5,547,835; 6,194,144; 6,043,031; 6,322,980; 6,340,566, and the like. Such technologies include, but are not limited to, allele-specific primer extension, allele-specific hybridization, allele-specific ligation, allele-specific enzymatic cleavage, mismatch detection using resolvase, and sequencing. These technologies can be combined with different signal detection technologies such as fluorescence, fluorescence resonance energy transfer, fluorescence polarization, luminescence and mass spectroscopy.

[038] In some embodiments of the method of the invention, the isolated DNA is combined with a PM1 extension primer and a PM2 extension primer, as defined below, in the presence of one or more SNP detection reagents, thereby creating a detection product. The

detection product is then examined to determine the presence or absence of a PM1 mutation or a PM2 mutation in the isolated DNA. As used herein, the term "SNP detection reagent" refers to any reagent that is part of any SNP technology, technique or kit that can be used to detect single nucleotide polymorphisms.

[039] In one embodiment, the template DNA is combined with a first extension primer which is suitable for detection of a PM1 mutation, a second extension primer suitable for detection of a PM2 mutation, and one or more SNP detection reagents. An "extension primer" is an oligonucleotide that binds to the target DNA upstream from the target mutation in the direction of extension. In accordance with the invention, a PM1 extension primer comprises an oligonucleotide corresponding to a PM1 mutation. Similarly, a PM2 extension primer comprises an oligonucleotide corresponding to a PM2 mutation. The extension primer will preferably have a length from about 12 nucleotides to about 100 nucleotides, and more preferably have a length from about 18 nucleotides to about 60 nucleotides.

[040] The extension primer may be chosen to bind substantially uniquely to a target sequence containing a PM1 or PM2 mutation under the conditions of primer extension, so that the sequence will normally be one that is conserved or the primer is long enough to bind in the presence of a few mismatches, usually fewer than about 10% mismatches. By knowing the sequence that is upstream from the PM1 or PM2 mutation, one can select a sequence that has a high G-C ratio, so as to have a high binding affinity for the target sequence. In addition, the extension primer should bind reasonably close to the PM1 or PM2 mutation, preferably not more than about 200 nucleotides away, more preferably not more than about 100 nucleotide away, and most preferably within 50 nucleotides. In a preferred embodiment, the extension primer binds between 1 and 5 nucleotides away from the PM1 or PM2 mutation.

[041] Both the PM1 extension primer and the PM2 extension primer described herein are preferred extension primers. In one embodiment of the present invention, the PM1 extension primer comprises a sequence as shown in SEQ ID NO:24, or any contiguous primer, noncontiguous primer or homologous primer thereof. In another or further embodiment of the present invention, the PM2 extension primer comprises a sequence as shown in SEQ ID NO:66, or any contiguous primer, noncontiguous primer or homologous primer thereof. The PM1 or PM2 primer can also comprise an RNA version of any of the aforementioned extension primers.

[042] The term "contiguous primer" refers to a polynucleotide sequence that contains at least a fragment of the polynucleotide sequence of SEQ ID NO:24, SEQ ID

NO:66, SEQ ID NO:23 or SEQ ID NO:65. In one embodiment, the contiguous primer contains a 5' or 3' fragment of SEQ ID NO:24, SEQ ID NO:66, SEQ ID NO:23 or SEQ ID NO:65 in addition to one or more nucleotides complementary to upstream or downstream PM1 or PM2 polynucleotide sequences. For example, a contiguous primer of the PM1 primer shown in SEQ ID NO:24 could comprise a nucleotide sequence of TAC ATCTTTGAAAGTGCCA (SEQ ID NO:89). The term "noncontiguous primer" refers to a sequence that is not contiguous with a PM1 or PM2 primer (i.e., a contiguous fragment of the PM1 or PM2 primer), but which sequence contains portions of a PM1 or PM2 primer sequence sufficient to provide the amplification or detection results obtained with SEQ ID NO:24, SEQ ID NO:66, SEQ ID NO:23 or SEQ ID NO:65. For example, with reference to Figure 1E, oligonucleotides having SEQ ID NOs: 5-21 are noncontiguous with the PM1 primer having SEQ ID NO:23. Finally, the term "homologous primer" refers to a polynucleotide sequence that is substantially homologous with SEQ ID NO:24, SEQ ID NO:66, SEQ ID NO:23 or SEQ ID NO:65 or a contiguous primer thereof. In a preferred embodiment, the contiguous, non-contiguous or homologous primer has the attributes of an extension primer as described above, and more preferably, binds immediately upstream or downstream from a PM1 or PM2 mutation.

[043] Substantially homologous primers included in the present invention are those that provide detection results in ranges similar to those obtained with the oligonucleotide sequence shown in SEQ ID NO:24, SEQ ID NO:66, SEQ ID NO:23 or SEQ ID NO:65. In a preferred embodiment, a primer substantially homologous to SEQ ID NO:24, SEQ ID NO:66, SEQ ID NO:23 or SEQ ID NO:65 is at least about 50-60%, preferably at least about 60-70%, and more preferably at least about 70-75%, 75-80%, 80-85%, 85-90% or 90-95%, and most preferably at least about 96%, 97%, 98%, 99% or more identical to an entire oligonucleotide sequence shown in SEQ ID NO:24, SEQ ID NO:66, SEQ ID NO:23 or SEQ ID NO:65.

[044] To determine the percent sequence identity of two polynucleotide sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in the sequence of one polynucleotide for optimal alignment with the other polynucleotide). The polynucleotides at corresponding positions are then compared. When a position in one sequence (e.g., a sequence of SEQ ID NO:24, SEQ ID NO:66, SEQ ID NO:23 or SEQ ID NO:65) is occupied by the same nucleotide as the corresponding position in the other sequence, then the molecules are identical at that position. Accordingly, the percent sequence identity between the two sequences is a function of the number of identical

positions shared by the sequences (i.e., percent sequence identity = numbers of identical positions/total numbers of positions x 100). For the purposes of the invention, the percent sequence identity between two nucleic acid or polypeptide sequences is determined using the Vector NTI 6.0 (PC) software package (InforMax, 7600 Wisconsin Ave., Bethesda, MD 20814). A gap opening penalty of 15 and a gap extension penalty of 6.66 are used for determining the percent identity of two nucleic acids. A gap opening penalty of 10 and a gap extension penalty of 0.1 are used for determining the percent identity of two polypeptides. All other parameters are set at the default settings. It is to be understood that for the purposes of determining sequence identity when comparing a DNA sequence to an RNA sequence, a thymidine nucleotide is equivalent to a uracil nucleotide.

[045] The methods described in the examples employ the coding sequences of the PM1 and PM2 mutations as templates, but the method works equally well with SNP detection assays using the non-coding sequence and the primers. For example, a PM1 extension primer with the non-coding strand as template ($^{5'}$TGTGTTACCGATGATCCCAA$^{3'}$; SEQ ID NO:23) and a PM2 extension primer with a non-coding strand as template ($^{5'}$TCTTGGGATGGTCATGCAAT$^{3'}$; SEQ ID NO:65) may be used with the ABIPrism SnaPshot assay available from Applied Biosystems (Foster City, CA).

[046] Prior to the detection steps, template DNA containing the PM1 and PM2 mutations may optionally be amplified using known methods. Amplification and creation of a DNA template can be achieved using any method known to those of skill in the art including PCR. The term "PCR" as used herein refers to the polymerase chain reaction method of DNA amplification. As will be understood by one of ordinary skill in the art, this term also includes any and all other methods known in the art for nucleic acid amplification requiring an amplification target, at least one primer and a polymerase.

[047] For example, either PM1 template DNA or PM2 template DNA may be amplified by combining the isolated genomic DNA with an appropriate primer set for the amplification of a polynucleotide sequence containing a PM1 or PM2 mutation. Each primer set consists of a forward primer and a reverse primer, each of which can be referred to as an "amplification primer." In one embodiment of the present invention, *AHAS1* and *AHAS3* template DNAs may be amplified using a single primer set wherein a first amplification primer comprises the sequence 5' GGC GTT TGG TGT TAG GTT TGA 3' (SEQ ID NO:90) and a second amplification primer comprises the sequence 5' CGT CTG GGA ACA ACC AAA AGT 3' (SEQ ID NO:91). Alternatively, an *AHAS1* template DNA may be separately

amplified using an *AHAS1*-specific forward primer 5' GGA AAG CTC GAG GCT TTC GCT 3' (SEQ ID NO: 92) and an *AHAS1/AHAS3* reverse primer 5' ATC ACC AGC TTC ATC TCT CAG T 3' (SEQ ID NO: 93). In this embodiment, an *AHAS3* template DNA may be separately amplified using an *AHAS3*-specific forward primer (5' GGA AAG CTC GAG GCG TTT GCG 3'; SEQ ID NO: 94) and the *AHAS1/ AHAS3* reverse primer (5' ATC ACC AGC TTC ATC TCT CAG T 3'; SEQ ID NO: 93).

[048] Those of ordinary skill will recognize that additional amplification primers may be prepared which are contiguous, noncontiguous or homologous primer to the amplification primers et forth above. The forward and reverse primers can also be an RNA version of any of the aforementioned amplification primers.

[049] The invention is further illustrated by the following examples, which are not to be construed in any way as imposing limitations upon the scope thereof.

EXAMPLES

Example 1

Isolation of genomic DNA from a Plant

[050] The DNA extraction procedure described below (Cheung *et al.*, 1993 PCR Methods and Applications 3:69-70) can be used for both fresh and lyophilized leaf tissues. If fresh leaf tissues are used, the Phenol and chloroform/isoamyl-alcohol extraction steps can be omitted.

[051] Two 5 mm diameter leaf discs made with a paper punch or the equivalent were taken from each leaf sample and immediately placed in 320 μl of sterile extraction buffer containing 200 mM Tris-HCl (pH 8.0), 70 mM EDTA, 2 M NaCl and 20 mM sodium metabisulfite. Leaves were then ground until no visible pieces of tissue remained. Cells were lysed with addition of 80 μl of 5% sodium sarcosyl to each tube and were incubated at 60 °C for an hour. After 15 minutes of centrifugation at 13,800 RPM, the supernatant was transferred to a fresh tube and an equal volume of buffer saturated phenol was added. The contents in the tubes were mixed by inverting a few times and were spun at 13,800 RPM for 5 minutes.

[052] The aqueous phase was then transferred into a fresh tube and an equal volume of chloroform/isoamyl alcohol (24:1 v/v) was added and mixed by inverting tubes a few times and then was spun at 13,800 RPM for 5 minutes. After transferring the aqueous phase to a fresh tube, 180 μl of filter-sterilized 10 M ammonium acetate and 400 μl of isopropanol were

then added and left at room temperature for 15 minutes for DNA precipitation. After centrifuging for 15 minutes at 13,800 RPM, the supernatant was removed the pellets were rinsed once in 70% EtOH and left to air dry. The DNA pellet was resuspended in 100 μl TE buffer with 0.01 mg/ml of RNase and a 9 μl aliquot of DNA was run on 0.8% agarose to check for quantity and quality.

Example 2

DNA Amplification and Clean-Up

[053] Preliminary testing showed that the primer pair, Primer 1 (5' GGC GTT TGG TGT TAG GTT TGA 3') (SEQ ID NO:90) and Primer 2 (5' CGT CTG GGA ACA ACC AAA AGT 3') (SEQ ID NO:91) could amplify in one PCR reaction sufficient amounts of both *AHAS1* and *AHAS3* sequences for both PM1 and PM2 tests. Each PCR reaction mixture was set up in a total volume of 75 μl containing 1X PCR buffer II (Perkin Elmer), 2.5 mM $MgCl_2$, 200 μM of each dNTP, 400 nM each of Primer 1 and Primer 2, 100 ng of DNA (or 4 μl of extracted DNA) and 3 units of AmpliTaq® DNA polymerase (Perkin Elmer). Amplification reactions were carried out in Perkin Elmer GeneAmp 9600 or 9700 PCR systems. The PCR program included an initial denaturing step at 94 °C, followed by 30 cycles of denaturation at 94 °C for 10 seconds, annealing at 56 °C for 15 seconds, and extension at 72 °C for 30 seconds with a final extension step of 5 minutes at 72 °C. An aliquot of the PCR product was checked on 1.4 % agarose for an expected product size of 1Kb.

[054] In the clean-up step, 50 μl of each PCR product was first treated with 10 units of CIP (calf intestinal phosphatase, NEW ENGLAND BioLabs Inc.) by incubating at 37 °C for 1 hour and then deactivating the enzyme by incubating at 72 °C for 15 minutes in Perkin Elmer GeneAmp 9700 PCR systems. Subsequently, the 50 μl aliquot was purified using the QIAquick™ 96 PCR Purification Kit (QIAGEN) and eluted in 50 μl ddH_2O. Samples were then placed in a Universal Vacuum System UVS400/Speed Vac® Plus SC110A (Savant) for approximately 1 hour or until the water in the sample completely evaporated. The CIP treated and purified PCR product was resuspended in ddH_2O at a concentration of approximately 50 ng/μl and was used as DNA templates for the primer extension reactions for detecting the PM1 and PM2 mutations.

Example 3

Primer Extension PCR for Detecting PM1 and PM2 Mutations using ABI PRISM®

[055] The ABI PRISM® SNaPshot ddNTP Primer Extension Kit was used on each DNA sample and to detect both the PM1 and the PM2 single nucleotide mutations. The mutation detecting primers are as follows: PM1 extension primer: 5' CAT CTT TGA AAG TGC CAC CA 3' (SEQ ID NO:24) for detection of the PM1 mutation and PM2 extension primer: 5' CTT TGT AGA ACC GAT CTT CC 3' (SEQ ID NO:66) for detection of the PM2 mutation. Primer extension reactions were performed with 100 ng of CIP treated and purified PCR amplified templates in a total volume of 10 µl with 100 nM of the appropriate mutation primer, SNaPshot Ready Reaction Premix as indicated by the manufacturer. Thermal cycling was performed in Perkin Elmer GeneAmp 9600 or 9700 PCR systems with conditions set for 25 cycles of denaturation at 96 °C for 10 seconds, annealing at 50 °C for 5 seconds and extension at 60 °C for 30 seconds. Post-extension treatment consisted of incubating the reaction mixture for 1 hour at 37 °C with 1 unit of calf intestinal phosphatase (NEW ENGLAND BioLabs Inc.) and the enzyme was inactivated at 72 °C for 15 minutes. Samples were then prepared for loading on an ABI PRISM® 3700 DNA Analyzer by adding 1µl of each post-extension treated reaction to 10 µl of deionized formamide, denatured at 95 °C for 5 minutes and then loaded and run using a GeneScan 5 Run Module. Data was collected and viewed using the ABI PRISM® GeneScan v. 3.5.1 software.

Example 4

Detection of PM1 and PM2 Mutations in B. napus using ABI PRISM®

[056] The PM1 test using the primer PM1 involves the extension of the next nucleotide to the primer sequence with the coding strand as the template. Thus, in the wildtype plant, here a *B. napus* cv. 'Topas' plant, the observed nucleotide should be "C" corresponding to the wildtype "G" in the codon "AGT" for Serine on the coding strand. When the test is done on the mutated PM1 *B. napus* plant, the observed nucleotide should be "T" corresponding to the mutated "A" in the codon "AAT" for Asparagine on the coding strand (Figure 2). The results obtained with the ABI PRISM® method showed exactly the predicted results. A mutated PM2 *B. napus* plant that did not contain the PM1 mutation was shown to provide the same results as the wildtype 'Topas' plant in the PM1 test. Therefore, the PM1 mutation was detected accurately in *B. napus* using the ABI PRISM® primer extension methodology.

[057] Similarly, the PM2 test using the primer PM2 involves the extension of the next nucleotide to the primer sequence with the coding strand as the template. Thus, in the wildtype plant, e.g. 'Topas', the observed nucleotide should be "C" corresponding to the wildtype "G" in the codon "TGG" for Tryptophan on the coding strand. When the test was done on the mutated PM2 *B. napus* plant, the observed nucleotide should be "A" corresponding to the mutated "T" in the codon "TTG" for Leucine on the coding strand (Figure 3). The results obtained with the present method showed exactly the predicted results. A mutated PM1 *B. napus* plant that does not have the PM2 mutation was shown to provide the same results as the wildtype 'Topas' plant in the PM2 test. Therefore, the PM2 mutation was detected accurately in *B. napus* using the ABI PRISM® primer extension methodology.

Example 5

Validation of ABI PRISM® PM1 and PM2 Detection Method

[058] In order to validate the use of the present method on plant materials with a genetic background different from the one used to develop the markers and the method (the *B. napus* 'Topas' plant), the PM1 and PM2 tests were performed to detect the presence or absence of the CLEARFIELD® trait on 24 doubled haploid (DH) (*i.e.*, homozygous) canola lines. These 24 lines were divided into four classes: PM1, PM2, PM1/PM2 and WT based on the results of survival after spraying with herbicide. The codes and classification of the DH lines are summarized in Figure 4, in which "GH Rating" means greenhouse rating on mortality: 0 means all plants survive after spraying and 85% means 85% of the plants died after spraying. Also included in Figure 4 are the three controls used in the validation tests: PM1, PM2 and WT, all from the *B. napus* 'Topas' var. used in Examples 2 through 4 for development of the PM1/PM2 assay. The amplification of the templates and the mutation tests were repeated three times for each DH line from Advanta Seeds and twice for the three control samples.

[059] The results of the PM1 and PM2 mutation tests are summarized in Figure 5. The plant number in Figure 5 corresponds to the plant number in Figure 4. Additionally, the peaks related to the mutations are in bold and in italics while the peaks that are not always present or present in various amounts in all the three replicates are in brackets. The "Expected Results" column reflects those results that are expected assuming that the amplification reaction using the primer pair *AHAS1/AHAS3* amplification primer of SEQ ID NO: 90 and the *AHAS1/AHAS3* amplification primer of SEQ ID NO: 91 amplified similar

amounts of both *AHAS1* and *AHAS3* sequences and that the PM1 extension primers will anneal also to the *AHAS3* sequence and the PM2 extension primers will anneal also to the *AHAS1* sequence.

[060] As shown in Figure 5, the observed results for both the PM1 and PM2 mutation tests agreed with the expected results for all six plants in the PM1/PM2 class. With the PM1 class, all six plants showed the PM1 mutation (as "T"). All of the wild-type plants showed the absence of either mutation. Therefore, with all three classes of plants, the present invention can correctly predict the presence or absence of the PM1 and PM2 mutations.

[061] The results for the PM2 class were more complicated. All the six plants of the PM2 classes were expected to have the PM2 mutation (i.e. an "A" with the PM2 mutation test). In fact, all the six plants did detect an "A" with the test throughout the three replicates. The PM2 class was expected to have the wild-type "C" for the PM1 mutation test. However, in the observed results, only plant #40 showed the wild-type "C", while each of the other five plants consistently showed a "T" for the PM1 mutation test, indicating the unexpected presence of the of the PM1 mutation . The control lines gave the expected results.

[062] It is believed that the discrepancy in the expected and actual results regarding the plants classified as containing only the PM2 mutation is due to misclassification under the herbicide spraying test and that this discrepancy reflects the superiority of the present invention. One advantage of using the present invention to identify the presence or the absence of PM1 or PM2 mutations over the herbicide spraying test is that the present invention can unequivocally tell whether the mutations are present in the genetic materials of the tested plants. Hence, the invention described herein presents a more reliable test which will not be influenced by other environmental factors.

[063] Using the present invention, one can easily tell apart the wild type plants from those with only the PM1 mutation and also differentiate between plants with only PM1 or PM2 mutations and those with both PM1 and PM2 mutations, which are particularly difficult to distinguish using the spraying test. With the prior art herbicide spraying test, a statistical number of plants of the same line need to be grown and sprayed to obtain meaningful results while with the present invention, fewer plants from the same line need to be tested. Since the methods of the present invention only require very small amount of leaf materials per line, another advantage of these methods is that they can be performed when the plants are very young, for example at the cotyledon stage. This advantage translates into savings in growth space and other costs.

Example 6

Detection of PM1 and PM2 Mutations in B. napus using PYROSEQUENCING PSQ ™ 96

[064] A second method to allow high throughput detection of the presence or absence of "PM1" and "PM2" mutations in *B. napus* was designed, the method comprising four steps:

1. Isolation of genomic DNA
2. Separation of *AHAS1* and *AHAS3* DNA template preparations by PCR with an *AHAS1*-specific forward primer paired with a biotinylated *AHAS1/AHAS3* reverse primer for *AHAS1* and an *AHAS3*-specific forward oligonucleotide primer paired with the same biotinylated *AHAS1/AHAS3* reverse primer for *AHAS3*
3. Isolation of single stranded DNA templates
4. PYROSEQUENCING™ reactions with PM1 sequencing primer for detecting the "PM1" mutation and PM2 sequencing primer for detecting the "PM2" mutations.

DNA Isolation

[065] The procedure set forth in Example 1 was used to isolate DNA from plants for analysis using the PYROSEQUENCING™ method.

DNA amplification

[066] For detection of the PM1 and PM2 mutations using the PYROSEQUENCING™ method, the best results were obtained when *AHAS1* and *AHAS3* sequences were separately amplified as templates. Therefore, two amplification reactions were first performed using different forward primers, *AHAS1*-specific forward primer for *AHAS1* (5' GGA AAG CTC GAG GCT TTC GCT 3'; SEQ ID NO:92) and *AHAS3*-specific forward primer for *ALSS3* (5' GGA AAG CTC GAG GCG TTT GCG 3'; SEQ ID NO: 94) but pairing with the same biotinylated reverse primer, *AHAS1/AHAS3* reverse primer (5' ATC ACC AGC TTC ATC TCT CAG T 3'; SEQ ID NO:93). Each PCR reaction was set up in a total volume of 30 µl containing 1X PCR buffer II (Applied Biosystems, Foster City, CA), 2.5 mM $MgCl_2$, 200 µM of each dNTP, 300 nM each of an *AHAS1*-specific forward primer and *AHAS1/AHAS3* reverse primer for *AHAS1* and an *AHAS3*-specific forward primer and *AHAS1/AHAS3* reverse primer for *AHAS3*, 5 ng of DNA and 1.25 units of AmpliTaq® Gold DNA polymerase (Applied Biosystems, Foster City, CA). Amplification reactions were carried out in Applied Biosystems GeneAmp 9600® or GeneAmp 9700® PCR systems. The PCR program includes an initial denaturing step at 94°C for 10 minutes, followed by 45 cycles of denaturation at 94°C for 10 seconds, annealing at 56°C for 15 seconds, and

extension at 72°C for 30 seconds with a final extension step of 10 minutes at 72°C. An aliquot of each PCR product was checked on 1% agarose for an expected product size of 1Kb.

Single strand template isolation and annealing of sequencing primers for detection of "PM1" and "PM2" mutations

[067] PCR amplified products were immobilized by mixing 25 µl of the PCR product with 150 ng of Dynabeads® M-280 Streptavidin (Dynal AS, Oslo, Norway) and 25 µl of 2X Binding-Washing buffer II pH 7.6 (PYROSEQUENCING™) and were incubated on an agitator at 65° for 30 minutes Using the PSQ 96 Sample Prep Tool, the beads carrying the biotinylated templates were then transferred and released into a PSQ 96 Plate containing 50 µl of 0.5 M NaOH per well and left to soak with gentle agitation for 1 minute. The beads now carrying the isolated biotinylated non-coding strands were then transferred into a second PSQ™ 96 Plate for a wash in 100 µl of 1X annealing buffer (PYROSEQUENCING™). Finally, annealing of the sequencing primers was done by transferring the beads into a third PSQ 96 Plate containing 44 µl of 1X annealing buffer (PYROSEQUENCING™) and either 10 pmol of PM1 sequencing primer (5' GTG TTA CCG ATG ATC C 3'; SEQ ID NO: 95) or 10 pmol of PM2 sequencing primer (5' GGG ATG GTC ATG CAA T 3'; SEQ ID NO: 96) for assaying the PM1 and PM2 mutations respectively. This third plate was then incubated at 94°C for 3 minutes and allowed to cool to room temperature for 5 to 10 minutes.

SNP detection using the PYROSEQUENCING (PSQ ™ 96) system

[068] The third PSQ 96 Plate containing PM1 or PM2 sequencing primers annealed to the non-coding biotinylated strands from each PCR product was loaded onto the PSQ ™ 96 system and the pyrosequencing run was carried out using the PSQ™ 96 Instrument Control module from the PSQ™ 96 SNP Software (version 1.2 AQ). The PSQ™ 96 SNP Entry module was used to enter the orders of dispensing nucleotides for both PM1 and PM2 detection (CTAGCTGTG for "PM1" detection and CTGCAGATC for "PM2" detection) while the PSQ™ 96 Evaluation module was used for viewing the results of pyrosequencing.

[069] The choice of the non-coding sequence as the template and the specific sequencing primers combinations for the "PM1" and "PM2" assay was the result of optimization of the process to produce unambiguous pyrograms that could infer the presence or absence of the mutations and whether they are present in the homozygous or heterozygous state.

Results of "PM1" and "PM2" tests using Pyrosequencing™

[070] Using the pyrosequencing technology platform for the "PM1" and "PM2" tests requires that the *AHAS1* and *AHAS3* sequences around the mutations to be amplified separately by specific PCR reactions. In the pyrosequencing technology, the incorporation of each nucleotide with the release of pyrophosphate during the primer extension reaction is coupled to the sulfurylase/luciferase system, which gives light signals proportional to the number of nucleotides incorporated at each elongation step. The results of the pyrosequencing reaction indicate the identity of the nucleotide sequences around the polymorphic site from which the nucleotide at the polymorphic site can be read. With the PM1 test, both *B. napus* 'Topas' and the *B. napus* 'PM2' line have the wildtype *AHAS1* sequence and the sequence extended from the PM1 sequencing primer is CAAGTGGTGG (SEQ ID NO:97); while for the mutant PM1 line, the extended sequence is CAAATGGTGG (SEQ ID NO:98) indicating the G→A PM1 mutation on the coding strand. With the PM2 test, both 'Topas' and the 'PM1' line have wildtype *AHAS3* sequence and the sequence extended from the PM2 sequencing primer is GGGAAGATC (SEQ ID NO:99); while for the mutant PM2 line, the extended sequence is TGGAAGATC (SEQ ID NO:100) indicating the G→T PM2 mutation on the coding strand. Thus both PM1 and PM2 mutations were detected accurately using the PYROSEQUENCING™ technology.

[071] Throughout this application, various publications are referenced. The disclosures of all of these publications and those references cited within those publications in are hereby incorporated by reference in their entireties. It should also be understood that the foregoing relates to preferred embodiments of the present invention and that numerous changes may be made therein without departing from the scope of the invention. On the contrary, it is to be clearly understood that resort may be had to various other embodiments, modifications, and equivalents thereof, which, after reading the description herein, may suggest themselves to those skilled in the art without departing from the spirit of the present invention and/or the scope of the appended claims.

CLAIMS

1. A method of assaying a plant for imidazolinone herbicide resistance conferred by the combination of a PM1 mutation of a *B. napus AHAS1* gene and a PM2 mutation of a *B. napus AHAS3* gene, the method comprising the steps of:

a) isolating genomic DNA from the plant;

b) determining the presence or absence of the PM1 mutation in the DNA; and

c) determining the presence or absence of the PM2 mutation in the DNA,

wherein the presence of the PM1 mutation and the PM2 mutation is indicative of commercially relevant imidazolinone tolerance in the plant.

2. The method of claim 1, wherein the plant is a *Brassica* species.

3. The method of claim 2, wherein the *Brassica* species is selected from the group consisting of *B. napus*, *B. campestris/rapa*, and *B. juncea.*

4. The method of claim 1, further comprising the step of amplifying the isolated DNA prior to determining the presence or absence of the PM1 and PM2 mutations.

5. The method of claim 1, wherein the determining steps are performed using a primer extension-based single nucleotide polymorphism detection method.

6. A PM1 primer extension oligonucleotide comprising a sequence selected from the group consisting of SEQ ID NO:5; SEQ ID NO:6; SEQ ID NO:7; SEQ ID NO:8; SEQ ID NO:9; SEQ ID NO:10; SEQ ID NO:11; SEQ ID NO:12; SEQ ID NO: 13; SEQ ID NO:14; SEQ ID NO:15; SEQ ID NO:16; SEQ ID NO:17; SEQ ID NO:18; SEQ ID NO: 19; SEQ ID NO:20; SEQ ID NO:21; SEQ ID NO:22; SEQ ID NO:23; SEQ ID NO:24; SEQ ID NO:25; SEQ ID NO:26; SEQ ID NO:27; SEQ ID NO: 28; SEQ ID NO:29; SEQ ID No;30; SEQ ID NO:31; SEQ ID NO:32; SEQ ID NO:33; SEQ ID NO:34; SEQ ID NO:35; SEQ ID NO:36; SEQ ID NO:37; SEQ ID NO:38; SEQ ID NO:39; SEQ ID NO:40; SEQ ID NO:41; SEQ ID NO:42 and SEQ ID NO:95.

7. A PM1 oligonucleotide comprising a sequence selected from the group consisting of SEQ ID NO:45 and SEQ ID NO:46.

8. A PM2 primer extension oligonucleotide comprising a sequence selected from the group consisting of SEQ ID NO:47; SEQ ID NO:48; SEQ ID NO:49; SEQ ID NO:50; SEQ ID NO:51; SEQ ID NO:52; SEQ ID NO:53; SEQ ID NO:54; SEQ ID NO:55; SEQ ID NO:56; SEQ ID NO:57; SEQ ID NO:58; SEQ ID NO:59; SEQ ID NO:60; SEQ ID NO:61; SEQ ID NO:62; SEQ ID NO:63; SEQ ID NO:64; SEQ ID NO:65; SEQ ID NO:66; SEQ ID NO:67; SEQ ID NO:68; SEQ ID NO:69; SEQ ID NO:70; SEQ ID NO:71; SEQ ID NO:72; SEQ ID NO:73; SEQ ID NO:74; SEQ ID NO:75; SEQ ID NO:76; SEQ ID NO:77; SEQ ID NO:78; SEQ ID NO:79; SEQ ID NO:80; SEQ ID NO:81; SEQ ID NO:82; SEQ ID NO:83; SEQ ID NO:84 and SEQ ID NO:96.

9. A PM2 oligonucleotide comprising a sequence selected from the group consisting of SEQ ID NO:87 and SEQ ID NO:88.

10. An oligonucleotide comprising a sequence selected from the group consisting of SEQ ID NO:43; SEQ ID NO:44; SEQ ID NO:85 and SEQ ID NO:86.

11. An amplification oligonucleotide comprising a sequence selected from the group consisting of SEQ ID NO:90; SEQ ID NO:91; SEQ ID NO:92; SEQ ID NO:93; and SEQ ID NO:94.

PM1-*AHAS1* nucleotide sequence(SEQ ID NO: 1) and translated amino acid sequence (SEQ ID NO: 101)

```
1    TCATCATCTCTCTCTCCTCAAACCATGGCGGCGGCAACATCGTCTTCTCCGATCTCCTTAACCGCTAAACCTTCTTCCAAATCCCCTCTACCCATTTCCA
                                   M  A  A  A  T  S  S  S  P  I  S  L  T  A  K  P  S  S  K  S  P  L  P  I  S

101  GATTCTCCCTTCCCTTCTCCTTAACCCCACAGAAAGACTCCTCCCGTCTCCACCGTCCTCTCGCCATCTCCGCCGTTCTCAACTCACCCGTCAATGTCGC
     R  F  S  L  P  F  S  L  T  P  Q  K  D  S  S  R  L  H  R  P  L  A  I  S  A  V  L  N  S  P  V  N  V  A

201  ACCTCCTTCCCCTGAAAAAACCGACAAGAACAAGACTTTCGTCTCCCGCTACGCTCCCGACGAGCCCCGCAAGGGTGCTGATATCCTCGTCGAAGCCCTC
      P  P  S  P  E  K  T  D  K  N  K  T  F  V  S  R  Y  A  P  D  E  P  R  K  G  A  D  I  L  V  E  A  L

301  GAGCGTCAAGGCGTCGAAACCGTCTTTGCTTATCCCGGAGGTGCTTCCATGGAGATCCACCAAGCCTTGACTCGCTCCTCCACCATCCGTAACGTCCTTC
      E  R  Q  G  V  E  T  V  F  A  Y  P  G  G  A  S  M  E  I  H  Q  A  L  T  R  S  S  T  I  R  N  V  L

401  CCCGTCACGAACAAGGAGGAGTCTTCGCCGCCGAGGGTTACGCTCGTTCCTCCGGCAAACCGGGAATCTGCATAGCCACTTCGGGTCCCGGAGCTACCAA
     P  R  H  E  Q  G  G  V  F  A  A  E  G  Y  A  R  S  S  G  K  P  G  I  C  I  A  T  S  G  P  G  A  T  N

501  CCTCGTCAGCGGGTTAGCAGACGCGATGCTTGACAGTGTTCCTCTTGTCGCCATTACAGGACAGGTCCCTCGCCGGATGATCGGTACTGACGCCTTCCAA
      L  V  S  G  L  A  D  A  M  L  D  S  V  P  L  V  A  I  T  G  Q  V  P  R  R  M  I  G  T  D  A  F  Q

601. GAGACACCAATCGTTGAGGTAACGAGGTCTATTACGAAACATAACTATCTGGTGATGGATGTTGATGACATACCTAGGATCGTTCAAGAAGCATTCTTTC
      E  T  P  I  V  E  V  T  R  S  I  T  K  H  N  Y  L  V  M  D  V  D  D  I  P  R  I  V  Q  E  A  F  F

701  TAGCTACTTCCGGTAGACCCGGACCGGTTTTGGTTGATGTTCCTAAGGATATTCAGCAGCAGCTTGCGATTCCTAACTGGGATCAACCTATGCGCTTGCC
     L  A  T  S  G  R  P  G  P  V  L  V  D  V  P  K  D  I  Q  Q  Q  L  A  I  P  N  W  D  Q  P  M  R  L  P

801  TGGCTACATGTCTAGGTTGCCTCAGCCWCCGGAAGTTTCTCAGTTAGGTCAGATCGTTAGGTTGATCTCGGAGTCTAAGAGGCCTGTTTTGTACGTTGGT
      G  Y  M  S  R  L  P  Q  X  P  E  V  S  Q  L  G  Q  I  V  R  L  I  S  E  S  K  R  P  V  L  Y  V  G

901  GGTGGAAGCTTGAACTCGAGTGAAGAACTGGGGAGATTTGTCGAGCTTACTGGGATCCCTGTTGCGAGTACGTTGATGGGGCTTGGCTCTTATCCTTGTA
      G  G  S  L  N  S  S  E  E  L  G  R  F  V  E  L  T  G  I  P  V  A  S  T  L  M  G  L  G  S  Y  P  C

1001 ACGATGAGTTGTCCCTGCAGATGCTTGGCATGCACGGGACTGTGTATGCTAACTACGCTGTGGAGCATAGTGATTTGTTGCTGGCGTTTGGTGTTAGGTT
     N  D  E  L  S  L  Q  M  L  G  M  H  G  T  V  Y  A  N  Y  A  V  E  H  S  D  L  L  L  A  F  G  V  R  F

1101 TGATGACCGTGTCACGGGAAAGCTCGAGGCTTTCGCTAGCAGGGCTAAAATTGTGCACATAGACATTGATTCTGCTGAGATTGGGAAGAATAAGACACCT
      D  D  R  V  T  G  K  L  E  A  F  A  S  R  A  K  I  V  H  I  D  I  D  S  A  E  I  G  K  N  K  T  P

1201 CACGTGTCTGTGTGTGGTGATGTAAAGCTGGCTTTGCAAGGGATGAACAAGGTTCTTGAGAACCGGGCGGAGGAGCTCAAGCTTGATTTCGGTGTTTGGA
      H  V  S  V  C  G  D  V  K  L  A  L  Q  G  M  N  K  V  L  E  N  R  A  E  E  L  K  L  D  F  G  V  W

1301 GGAGTGAGTTGAGCGAGCAGAAACAGAAGTTCCCTTTGAGCTTCAAAACGTTTGGAGAAGCCATTCCTCCGCAGTACGCGATTCAGATCCTCGACGAGCT
     R  S  E  L  S  E  Q  K  Q  K  F  P  L  S  F  K  T  F  G  E  A  I  P  P  Q  Y  A  I  Q  I  L  D  E  L

1401 AACCGAAGGGAAGGCAATTATCAGTACTGGTGTTGGACAGCGTCAGATGTGGGCGGCGCAGTTTTACAAGTACAGGAAGCCGAGACAGTGGCTGTCGTCA
      T  E  G  K  A  I  I  S  T  G  V  G  Q  R  Q  M  W  A  A  Q  F  Y  K  Y  R  K  P  R  Q  W  L  S  S

1501 TCAGGCCTCGGAGCTATGGGTTTTGGACTTCCTGCTGCGATTGGAGCGTCTGTGGCGAACCCTGATGCGATTGTTGTGGATATTGACGGTGATGGAAGCT
      S  G  L  G  A  M  G  F  G  L  P  A  A  I  G  A  S  V  A  N  P  D  A  I  V  V  D  I  D  G  D  G  S

1601 TCATAATGAACGTTCAAGAGCTGGCCACAATCCGTGTAGAGAATCTTCCTGTGAAGATACTCTTGTTAAACAACCAGCATCTTGGGATGGTCATGCAATG
     F  I  M  N  V  Q  E  L  A  T  I  R  V  E  N  L  P  V  K  I  L  L  L  N  N  Q  H  L  G  M  V  M  Q  W

1701 GGAAGATCGGTTCTACAAAGCTAACAGAGCTCACACTTATCTCGGGGACCCGGCAAGGGAGAACGAGATCTTCCCTAACATGCTGCAGTTTGCAGGAGCT
      E  D  R  F  Y  K  A  N  R  A  H  T  Y  L  G  D  P  A  R  E  N  E  I  F  P  N  M  L  Q  F  A  G  A

1801 TGCGGGATTCCAGCTGCGAGAGTGACGAAGAAAGAAGAACTCCGAGAAGCTATTCAGACAATGCTGGATACACCAGGACCATACCTGTTGGATGTGATAT
      C  G  I  P  A  A  R  V  T  K  K  E  E  L  R  E  A  I  Q  T  M  L  D  T  P  G  P  Y  L  L  D  V  I

1901 GTCCGCACCAAGAACATGTGTTACCGATGATCCCAAATGGTGGCACTTTCAAAGATGTAATAACAGAAGGGGATGGTCGCACTAAGTACTGAGAGATTMA
     C  P  H  Q  E  H  V  L  P  M  I  P  N  G  G  T  F  K  D  V  I  T  E  G  D  G  R  T  K  Y

2001 GCTGGTGATCGATCATATGGTAAAAGACTTAGTTTCAGTTTCCAGTTTCTTTTGTGTGGTAATTTGGGTTTGTCAGTTGTTGT
```

FIG. 1A

PM2-*AHAS3* nucleotide sequence (SEQ ID NO:2) and translated amino acid sequence (SEQ ID NO: 102)

```
1    TTCATCATMTCTCTCTCATTTCTCTCTCTCTCATCTAACCATGGCGGCGGCAACATCGTCTTCTCCGATCTCCTTAACCGCTAAACCTTCTTCCAAAT
                                             M  A  A  A  T  S  S  S  P  I  S  L  T  A  K  P  S  S  K

101  CCCCTCTACCCATTTCCAGATTCTCCCTTCCCTTCTCCTTAACCCCACAGAAACCCTCCTCCCGTCTCCACCGTCCACTCGCCATCTCCGCCGTTCTCAA
     S  P  L  P  I  S  R  F  S  L  P  F  S  L  T  P  Q  K  P  S  S  R  L  H  R  P  L  A  I  S  A  V  L  N

201  CTCACCCGTCAATGTCGCACCTGAAAAAACCGACAAGATCAAGACTTTCATCTCCCGCTACGCTCCCGACGAGCCCCGCAAGGGTGCTGATATCCTCGTG
      S  P  V  N  V  A  P  E  K  T  D  K  I  K  T  F  I  S  R  Y  A  P  D  E  P  R  K  G  A  D  I  L  V

301  GAAGCCCTCGAGCGTCAAGGCGTCGAAACCGTCTTCGCTTATCCCGGAGGTGCCTCCATGGAGATCCACCAAGCCTTGACTCGCTCCTCCACCATCCGTA
      E  A  L  E  R  Q  G  V  E  T  V  F  A  Y  P  G  G  A  S  M  E  I  H  Q  A  L  T  R  S  S  T  I  R

401  ACGTCCTCCCCCGTCACGAACAAGGAGGAGTCTTCGCCGCCGAGGGTTACGCTCGTTCCTCCGGCAAACCGGGAATCTGCATAGCCACTTCGGGTCCCGG
     N  V  L  P  R  H  E  Q  G  G  V  F  A  A  E  G  Y  A  R  S  S  G  K  P  G  I  C  I  A  T  S  G  P  G

501  AGCTACCAACCTCGTCAGCGGGTTAGCCGACGCGATGCTTGACAGTGTTCCTCTCGTCGCCATCACAGGACAGGTCCCTCGCCGGATGATCGGTACTGAC
      A  T  N  L  V  S  G  L  A  D  A  M  L  D  S  V  P  L  V  A  I  T  G  Q  V  P  R  R  M  I  G  T  D

601  GCGTTCCAAGAGACGCCAATCGTTGAGGTAACGAGGTCTATTACGAAACATAACTATCTGGTGATGGATGTTGATGACATACCTAGGATCGTTCAAGAAG
      A  F  Q  E  T  P  I  V  E  V  T  R  S  I  T  K  H  N  Y  L  V  M  D  V  D  D  I  P  R  I  V  Q  E

701  CATTCTTTCTAGCTACTTCCGGTAGACCCGGACCGGTTTTGGTTGATGTTCCTAAGGATATTCAGCAGCAGCTTGCGATTCCTAACTGGGATCAACCTAT
     A  F  F  L  A  T  S  G  R  P  G  P  V  L  V  D  V  P  K  D  I  Q  Q  Q  L  A  I  P  N  W  D  Q  P  M

801  GCGCTTGCCTGGCTACATGTCTAGGCTGCCTCAGCCACCGGAAGTTTCTCAGTTAGGCCAGATCGTTAGGTTGATCTCGGAGTCTAAGAGGCCTGTTTTG
      R  L  P  G  Y  M  S  R  L  P  Q  P  P  E  V  S  Q  L  G  Q  I  V  R  L  I  S  E  S  K  R  P  V  L

901  TACGTTGGTGGTGGAAGCTTGAACTCGAGTGAAGAACTGGGGAGATTTGTCGAGCTTACTGGGATCCCTGTTGCGAGTACGCTGATGGGGCTTGGCTCTT
      Y  V  G  G  G  S  L  N  S  S  E  E  L  G  R  F  V  E  L  T  G  I  P  V  A  S  T  L  M  G  L  G  S

1001 ATCCTTGTAACGATGAGTTGTCCCTGCAGATGCTTGGCATGCACGGGACTGTGTATGCTAACTACGCTGTGGAGCATAGTGATTTGTTGCTGGCGTTTGG
     Y  P  C  N  D  E  L  S  L  Q  M  L  G  M  H  G  T  V  Y  A  N  Y  A  V  E  H  S  D  L  L  L  A  F  G

1101 TGTTAGGTTTGATGACCGTGTCACGGGAAAGCTCGAGGCGTTTGCGAGCAGGGCTAAGATTGTGCACATAGACATTGATTCTGCTGAGATTGGGAAGAAT
      V  R  F  D  D  R  V  T  G  K  L  E  A  F  A  S  R  A  K  I  V  H  I  D  I  D  S  A  E  I  G  K  N

1201 AAGACACCTCACGTGTCTGTGTGTGGTGATGTAAAGCTGGCTTTGCAAGGGATGAACAAGGTTCTTGAGAACCGGGCGGAGGAGCTCAAGCTTGATTTCG
      K  T  P  H  V  S  V  C  G  D  V  K  L  A  L  Q  G  M  N  K  V  L  E  N  R  A  E  E  L  K  L  D  F

1301 GTGTTTGGAGGAGTGAGTTGAGCGAGCAGAAACAGAAGTTCCCGTTGAGCTTCAAAACGTTTGGAGAAGCCATTCCTCCGCAGTACGCGATTCAGGTCCT
     G  V  W  R  S  E  L  S  E  Q  K  Q  K  F  P  L  S  F  K  T  F  G  E  A  I  P  P  Q  Y  A  I  Q  V  L

1401 AGACGAGCTAACCCAAGGGAAGGCAATTATCAGNACTGGTGTTGGACAGCATCAGATGTGGGCGGCGCAGTTTTACAAGTACAGGAAGCCGAGGCAGTGG
      D  E  L  T  Q  G  K  A  I  I  X  T  G  V  G  Q  H  Q  M  W  A  A  Q  F  Y  K  Y  R  K  P  R  Q  W

1501 CTGTCGTCCTCAGGACTCGGAGCTATGGGTTTCGGACTTCCTGCTGCGATTGGAGCGTCTGTGGCGAACCCTGATGCGATTGTTGTGGACATTGACGGTG
      L  S  S  S  G  L  G  A  M  G  F  G  L  P  A  A  I  G  A  S  V  A  N  P  D  A  I  V  V  D  I  D  G

1601 ATGGAAGCTTCATAATGAACGTTCAAGAGCTGGCCACAATCCGTGTAGAGAATCTTCCTGTGAAGATACTCTTGTTAAACAACCAGCATCTTGGGATGGT
     D  G  S  F  I  M  N  V  Q  E  L  A  T  I  R  V  E  N  L  P  V  K  I  L  L  L  N  N  Q  H  L  G  M  V

1701 CATGCAATTTGGAAGATCGGTTCTACAAAGCTAACAGAGCTCACACTTATCTCGGGGACCCGGCAAGGGAGAACGAGATCTTCCCTAACATGCTGCAGTTT
      M  Q  L  E  D  R  F  Y  K  A  N  R  A  H  T  Y  L  G  D  P  A  R  E  N  E  I  F  P  N  M  L  Q  F

1801 GCAGGAGCTTGCGGGATTCCAGCTGCGAGAGTGACGAAGAAAGAAGAACTCCGAGAAGCTATTCAGACAATGCTGGATACACCTGGACCGTACCTGTTGG
      A  G  A  C  G  I  P  A  A  R  V  T  K  K  E  E  L  R  E  A  I  Q  T  M  L  D  T  P  G  P  Y  L  L

1901 ATGCCATCTGTCCGCACCAAGAACATGTGTTACCGATGATCCCAAGTGGTGGCACTTTCAAAGATGTAATAACCGAAGGGGATGGTCGCACTAAGTACTG
      D  A  I  C  P  H  Q  E  H  V  L  P  M  I  P  S  G  G  T  F  K  D  V  I  T  E  G  D  G  R  T  K  Y

2001 AGAGATGAAGCTGGTGATCCATCGTATGGTAAAAGACTTAGTTTCAGTTTTCAGTTTCTTTTGTGTGGTAATTTGGGTTTGTCAGTTGTTGTTYTGCTTT

2101 TGGTTTGTTCCCNKAC
```

FIG. 1B

SUBSTITUTE SHEET (RULE 26)

T-*AHAS1* nucleotide sequence (SEQ ID NO:3) and translated amino acid sequence (SEQ ID NO: 103)

```
1    TCATCATCTCTCTCTCCTCTAACCATGGCGGCGGCAACATCGTCTTCTCCGATCTCCTTAACCGCTAAACCTTCTTCCAAATCCCCTCTACCCATTTCCA
                                M  A  A  A  T  S  S  S  P  I  S  L  T  A  K  P  S  S  K  S  P  L  P  I  S

101  GATTCTCCCTTCCCTTCTCCTTAACCCCACAGAAAGACTCCTCCCGTCTCCACCGTCCTCTCGCCATCTCCGCCGTTCTCAACTCACCCGTCAATGTCGC
     R  F  S  L  P  F  S  L  T  P  Q  K  D  S  S  R  L  H  R  P  L  A  I  S  A  V  L  N  S  P  V  N  V  A

201  ACCTCCTTCCCCTGAAAAAACCGACAAGAACAAGACTTTCGTCTCCCGCTACGCTCCCGACGAGCCCCGCAAGGGTGCTGATATCCTCGTCGAAGCCCTC
       P  P  S  P  E  K  T  D  K  N  K  T  F  V  S  R  Y  A  P  D  E  P  R  K  G  A  D  I  L  V  E  A  L

301  GAGCGTCAAGGCGTCGAAACCGTCTTTGCTTATCCCGGAGGTGCTTCCATGGAGATCCACCAAGCCTTGACTCGCTCCTCCACCATCCGTAACGTCCTTC
      E  R  Q  G  V  E  T  V  F  A  Y  P  G  G  A  S  M  E  I  H  Q  A  L  T  R  S  S  T  I  R  N  V  L

401  CCCGTCACGAACAAGGAGGAGTCTTCGCCGCCGAGGGTTACGCTCGTTCCTCCGGCAAACCGGGAATCTGCATAGCCACTTCGGGTCCCGGAGCTACCAA
     P  R  H  E  Q  G  G  V  F  A  A  E  G  Y  A  R  S  S  G  K  P  G  I  C  I  A  T  S  G  P  G  A  T  N

501  CCTCGTCAGCGGGTTAGCAGACGCGATGCTTGACAGTGTTCCTCTTGTCGCCATTACAGGACAGGTCCCTCGCCGGATGATCGGTACTGACGCCTTCCAA
      L  V  S  G  L  A  D  A  M  L  D  S  V  P  L  V  A  I  T  G  Q  V  P  R  R  M  I  G  T  D  A  F  Q

601  GAGACACCAATCGTTGAGGTAACGAGGTCTATTACGAAACATAACTATTTGGTGATGGATGTTGATGACATACCTAGGATCGTTCAAGAAGCTTTCTTTC
      E  T  P  I  V  E  V  T  R  S  I  T  K  H  N  Y  L  V  M  D  V  D  D  I  P  R  I  V  Q  E  A  F  F

701  TAGCTACTTCCGGTAGACCCGGACCGGTTTTGGTTGATGTTCCTAAGGATATTCAGCAGCAGCTTGCGATTCCTAACTGGGATCAACCTATGCGCTTACC
     L  A  T  S  G  R  P  G  P  V  L  V  D  V  P  K  D  I  Q  Q  Q  L  A  I  P  N  W  D  Q  P  M  R  L  P

801  TGGCTACATGTCTAGGTTGCCTCAGCCTCCGGAAGTTTCTCAGTTAGGTCAGATCGTTAGGTTGATCTCGGAGTCTAAGAGGCCTGTTTTGTACGTTGGT
       G  Y  M  S  R  L  P  Q  P  P  E  V  S  Q  L  G  Q  I  V  R  L  I  S  E  S  K  R  P  V  L  Y  V  G

901  GGTGGAAGCTTGAACTCGAGTGAAGAACTGGGGAGATTTGTCGAGCTTACTGGGATCCCCGTTGCGAGTACTTTGATGGGGCTTGGCTCTTATCCTTGTA
      G  G  S  L  N  S  S  E  E  L  G  R  F  V  E  L  T  G  I  P  V  A  S  T  L  M  G  L  G  S  Y  P  C

1001 ACGATGAGTTGTCCCTGCAGATGCTTGGCATGCACGGGACTGTGTATGCTAACTACGCTGTGGAGCATAGTGATTTGTTGCTGGCGTTTGGTGTTAGGTT
     N  D  E  L  S  L  Q  M  L  G  M  H  G  T  V  Y  A  N  Y  A  V  E  H  S  D  L  L  L  A  F  G  V  R  F

1101 TGATGACCGTGTCACGGGAAAGCTCGAGGCTTTCGCTAGCAGGGCTAAAATTGTGCACATAGACATTGATTCTGCTGAGATTGGGAAGAATAAGACACCT
       D  D  R  V  T  G  K  L  E  A  F  A  S  R  A  K  I  V  H  I  D  I  D  S  A  E  I  G  K  N  K  T  P

1201 CACGTGTCTGTGTGTGGTGATGTAAAGCTGGCTTTGCAAGGGATGAACAAGGTTCTTGAGAACCGGGCGGAGGAGCTCAAGCTTGATTTCGGTGTTTGGA
      H  V  S  V  C  G  D  V  K  L  A  L  Q  G  M  N  K  V  L  E  N  R  A  E  E  L  K  L  D  F  G  V  W

1301 GGAGTGAGTTGAGCGAGCAGAAACAGAAGTTCCCTTTGAGCTTCAAAACGTTTGGAGAAGCCATTCCTCCGCAGTACGCGATTCAGATCCTCGACGAGCT
     R  S  E  L  S  E  Q  K  Q  K  F  P  L  S  F  K  T  F  G  E  A  I  P  P  Q  Y  A  I  Q  I  L  D  E  L

1401 AACCGAAGGGAAGGCAATTATCAGTACTGGTGTTGGACAGCATCAGATGTGGGCGGCGCAGTTTTACAAGTACAGGAAGCCGAGACAGTGGCTGTCGTCA
       T  E  G  K  A  I  I  S  T  G  V  G  Q  H  Q  M  W  A  A  Q  F  Y  K  Y  R  K  P  R  Q  W  L  S  S

1501 TCAGGCCTCGGAGCTATGGGTTTTGGACTTCCTGCTGCGATTGGAGCGTCTGTGGCGAACCCTGATGCGATTGTTGTGGATATTGACGGTGATGGAAGCT
      S  G  L  G  A  M  G  F  G  L  P  A  A  I  G  A  S  V  A  N  P  D  A  I  V  V  D  I  D  G  D  G  S

1601 TCATAATGAACGTTCAAGAGCTGGCCACAATCCGTGTAGAGAATCTTCCTGTGAAGATACTCTTGTTAAACAACCAGCATCTTGGGATGGTCATGCAATG
     F  I  M  N  V  Q  E  L  A  T  I  R  V  E  N  L  P  V  K  I  L  L  L  N  N  Q  H  L  G  M  V  M  Q  W

1701 GGAAGATCGGTTCTACAAAGCTAACAGAGCTCACACTTATCTCGGGGACCCGGCAAGGGAGAACGAGATCTTCCCTAACATGCTGCAGTTTGCAGGAGCT
       E  D  R  F  Y  K  A  N  R  A  H  T  Y  L  G  D  P  A  R  E  N  E  I  F  P  N  M  L  Q  F  A  G  A

1801 TGCGGGATTCCAGCTGCGAGAGTGACGAAGAAAGAAGAACTCCGAGAAGCTATTCAGACAATGCTGGATACACCAGGACCATACCTGTTGGATGTGATAT
      C  G  I  P  A  A  R  V  T  K  K  E  E  L  R  E  A  I  Q  T  M  L  D  T  P  G  P  Y  L  L  D  V  I

1901 GTCCGCACCAAGAACATGTGTTACCGATGATCCCAAGTGGTGGCACTTTCAAAGATGTAATAACAGAAGGGGATGGTCGCACTAAGTACTGAGAGATGAA
     C  P  H  Q  E  H  V  L  P  M  I  P  S  G  G  T  F  K  D  V  I  T  E  G  D  G  R  T  K  Y

2001 GCTGGTGATCGATCATATGGTAAAAGACTTAGTTTCAGTTTCCAGTTTCTTTTGTGTGGTAATTTGGGTTTGTCAGTTGTTGT
```

FIG. 1C

T-*AHAS3* (SEQ ID NO: 4) nucleotide sequence and translated amino acid sequence (SEQ ID NO: 104)

```
1    TTMMACATCTCTCTCTCATTNCACTCTCTCCCTCATCTAACCATGGCGGCGGCAACATCGCCTTCTCCGATCTCCTTAACCGCTAAACCTTCTTCCAAAT
                                                 M  A  A  A  T  S  P  S  P  I  S  L  T  A  K  P  S  S  K
101  CCCCTCTACCCATTTCCAGATTCTCCCTTCCCTTCTCCTTAACCCCACAGAAACCCTCCTCCCGTCTCCACCGTCCACTCGCCATCTCCGCCGTTCTCAA
     S  P  L  P  I  S  R  F  S  L  P  F  S  L  T  P  Q  K  P  S  S  R  L  H  R  P  L  A  I  S  A  V  L  N
201  CTCACCCGTCAATGTCGCACCTGAAAAAACCGACAAGATCAAGACTTTCATCTCCCGCTACGCTCCCGACGAGCCCCGCAAGGGTGCTGATATCCTCGTG
       S  P  V  N  V  A  P  E  K  T  D  K  I  K  T  F  I  S  R  Y  A  P  D  E  P  R  K  G  A  D  I  L  V
301  GAAGCCCTCGAGCGTCAAGGCGTCGAAACCGTCTTCGCTTATCCCGGAGGTGCCTCCATGGAGATCCACCAAGCCTTGACTCGCTCCTCCACCATCCGTA
      E  A  L  E  R  Q  G  V  E  T  V  F  A  Y  P  G  G  A  S  M  E  I  H  Q  A  L  T  R  S  S  T  I  R
401  ACGTCCTCCCCCGTCACGAACAAGGAGGAGTCTTCGCCGCCGAGGGTTACGCTCGTTCCTCCGGCAAACCGGGAATCTGCATAGCCACTTCGGGTCCCGG
     N  V  L  P  R  H  E  Q  G  G  V  F  A  A  E  G  Y  A  R  S  S  G  K  P  G  I  C  I  A  T  S  G  P  G
501  AGCTACCAACCTCGTCAGCGGGTTAGCCGACGCGATGCTTGACAGTGTTCCTCTCGTCGCCATCACAGGACAGGTCCCTCGCCGGATGATCGGTACTGAC
       A  T  N  L  V  S  G  L  A  D  A  M  L  D  S  V  P  L  V  A  I  T  G  Q  V  P  R  R  M  I  G  T  D
601  GCGTTCCAAGAGACGCCAATCGTTGAGGTAACGAGGTCTATTACGAAACATAACTATCTGGTGATGGATGTTGATGACATACCTAGGATCGTTCAAGAAG
      A  F  Q  E  T  P  I  V  E  V  T  R  S  I  T  K  H  N  Y  L  V  M  D  V  D  D  I  P  R  I  V  Q  E
701  CATTCTTTCTAGCTACTTCCGGTAGACCCGGACCGGTTTTGGTTGATGTTCCTAAGGATATTCAGCAGCAGCTTGCGATTCCTAACTGGGATCAACCTAT
     A  F  F  L  A  T  S  G  R  P  G  P  V  L  V  D  V  P  K  D  I  Q  Q  Q  L  A  I  P  N  W  D  Q  P  M
801  GCGCTTGCCTGGCTACATGTCTAGGCTGCCTCAGCCACCGGAAGTTTCTCAGTTAGGCCAGATCGTTAGGTTGATCTCGGAGTCTAAGAGGCCTGTTTTG
       R  L  P  G  Y  M  S  R  L  P  Q  P  P  E  V  S  Q  L  G  Q  I  V  R  L  I  S  E  S  K  R  P  V  L
901  TACGTTGGTGGTGGAAGCTTGAACTCGAGTGAGGAACTGGGGAGATTTGTCGAGCTTACTGGGATCCCTGTTGCGAGTACGTTGATGGGGCTTGGCTCTT
      Y  V  G  G  G  S  L  N  S  S  E  E  L  G  R  F  V  E  L  T  G  I  P  V  A  S  T  L  M  G  L  G  S
1001 ATCCTTGTAACGATGAGTTGTCCCTGCAGATGCTTGGCATGCACGGGACTGTGTATGCTAACTACGCTGTGGAGCATAGTGATTTGTTGCTGGCGTTTGG
     Y  P  C  N  D  E  L  S  L  Q  M  L  G  M  H  G  T  V  Y  A  N  Y  A  V  E  H  S  D  L  L  L  A  F  G
1101 TGTTAGGTTTGATGACCGTGTCACGGGAAAGCTCGAGGCGTTTGCGAGCAGGGCTAAGATTGTGCACATAGACATTGATTCTGCTGAGATTGGGAAGAAT
       V  R  F  D  D  R  V  T  G  K  L  E  A  F  A  S  R  A  K  I  V  H  I  D  I  D  S  A  E  I  G  K  N
1201 AAGACACCTCACGTGTCTGTGTGTGGTGATGTAAAGCTGGCTTTGCAAGGGATGAACAAGGTTCTTGAGAACCGGGCGGAGGAGCTCAAGCTTGATTTCG
      K  T  P  H  V  S  V  C  G  D  V  K  L  A  L  Q  G  M  N  K  V  L  E  N  R  A  E  E  L  K  L  D  F
1301 GTGTTTGGAGGAGTGAGTTGAGCGAGCAGAAACAGAAGTTCCCGTTGAGCTTCAAAACGTTTGGAGAAGCCATTCCTCCGCAGTACGCGATTCAGGTCCT
     G  V  W  R  S  E  L  S  E  Q  K  Q  K  F  P  L  S  F  K  T  F  G  E  A  I  P  P  Q  Y  A  I  Q  V  L
1401 AGACGAGCTAACCCAAGGGAAGGCAATTATCAGTACTGGTGTTGGACAGCATCAGATGTGGGCGGCGCAGTTTTACAAGTACAGGAAGCCGAGGCAGTGG
       D  E  L  T  Q  G  K  A  I  I  S  T  G  V  G  Q  H  Q  M  W  A  A  Q  F  Y  K  Y  R  K  P  R  Q  W
1501 CTGTCGTCCTCAGGACTCGGAGCTATGGGTTTCGGACTTCCTGCTGCGATTGGAGCGTCTGTGGCGAACCCTGATGCGATTGTTGTGGACATTGACGGTG
      L  S  S  S  G  L  G  A  M  G  F  G  L  P  A  A  I  G  A  S  V  A  N  P  D  A  I  V  V  D  I  D  G
1601 ATGGAAGCTTCATAATGAACGTTCAAGAGCTGGCCACAATCCGTGTAGAGAATCTTCCTGTGAAGATACTCTTGTTAAACAACCAGCATCTTGGGATGGT
     D  G  S  F  I  M  N  V  Q  E  L  A  T  I  R  V  E  N  L  P  V  K  I  L  L  L  N  N  Q  H  L  G  M  V
1701 CATGCAATGGGAAGATCGGTTCTACAAAGCTAACAGAGCTCACACTTATCTCGGGGACCCGGCAAGGGAGAACGAGATCTTCCCTAACATGCTGCAGTTT
       M  Q  W  E  D  R  F  Y  K  A  N  R  A  H  T  Y  L  G  D  P  A  R  E  N  E  I  F  P  N  M  L  Q  F
1801 GCAGGAGCTTGCGGGATTCCAGCTGCGAGAGTGACGAAGAAAGAAGAACTCCGAGAAGCTATTCAGACAATGCTGGATACACCTGGACCGTACCTGTTGG
      A  G  A  C  G  I  P  A  A  R  V  T  K  K  E  E  L  R  E  A  I  Q  T  M  L  D  T  P  G  P  Y  L  L
1901 ATGTCATCTGTCCGCACCAAGAACATGTGTTACCGATGATCCCAAGTGGTGGCACTTTCGAAGATGTAATAACCGAAGGGGATGGTCGCACTAAGTACTG
     D  V  I  C  P  H  Q  E  H  V  L  P  M  I  P  S  G  G  T  F  E  D  V  I  T  E  G  D  G  R  T  K  Y
2001 AGAGATGAAGCTGGTGATCCATCATATGGTAAAAGACTTAGTTTCAGTTTACAGTTTCTTTTGTGTGGTAATTTGGGTTTGTCAGTTGTTGTTCTGCTTT

2101 TGGTTTGTTCCCWKAC
```

FIG. 1D

List of oligonucleotides suitable for detection of the PM1 and PM2 mutations. All oligonuceotides are in 5′-3′ orientation.

SEQ ID NO.	AHAS1-PM1 forward	SEQ ID NO.	AHAS1-PM1 reverse	SEQ ID NO.	AHAS3-PM2 forward	SEQ ID NO.	AHAS3-PM2 reverse
5	TTATCTCGGGGACCCGGCAA	24	CATCTTTGAAAGTGCCACCA	47	CTCAGGACTCGGAGCTATGG	66	CTTTGTAGAACCGATCTTCC
6	GACCCGGCAAGGGAGAACGA	25	TCTGTTATTACATCTTTGAA	48	GGAGCTATGGGTTTCGGACT	67	GCTCTGTTAGCTTTGTAGAA
7	GGGAGAACGAGATCTTCCCT	26	ACCATCCCCTTCTGTTATTA	49	GTTTCGGACTTCCTGCTGCG	68	ATAAGTGTGAGCTCTGTTAG
8	GATCTTCCCTAACATGCTGC	27	ACTTAGTGCGACCATCCCCT	50	TCCTGCTGCGATTGGAGCGT	69	GGTCCCCGAGATAAGTGTGA
9	AACATGCTGCAGTTTGCAGG	28	ATCTCTCAGTACTTAGTGCG	51	ATTGGAGCGTCTGTGGCGAA	70	TCCCTTGCCGGGTCCCCGAG
10	AGTTTGCAGGAGCTTGCGGG	29	CACCAGCTTCATCTCTCAGT	52	CTGTGGCGAACCCTGATGCG	71	GATCTCGTTCTCCCTTGCCG
11	AGCTTGCGGGATTCCAGCTG	30	TATGATCGATCACCAGCTTC	53	CCCTGATGCGATTGTTGTGG	72	TGTTAGGGAAGATCTCGTTC
12	ATTCCAGCTGCGAGAGTGAC	31	TCTTTTACCATATGATCGAT	54	ATTGTTGTGGACATTGACGG	73	AACTGCAGCATGTTAGGGAA
13	CGAGAGTGACGAAGAAAGAA	32	TGAAACTAAGTCTTTTACCA	55	ACATTGACGGTGATGGAAGC	74	AGCTCCTGCAAACTGCAGCA
14	GAAGAAAGAAGAACTCCGAG	33	AACTGGAAACTGAAACTAAG	56	TGATGGAAGCTTCATAATGA	75	GAATCCCGCAAGCTCCTGCA
15	GAACTCCGAGAAGCTATTCA	34	ACACAAAAGAAACTGGAAAC	57	TTCATAATGAACGTTCAAGA	76	CTCGCAGCTGGAATCCCGCA
16	AAGCTATTCAGACAATGCTG	35	CCAAATTACCACACAAAAGA	58	ACGTTCAAGAGCTGGCCACA	77	CTTCGTCACTCTCGCAGCTG
17	GACAATGCTGGATACACCAG	36	ACTGACAAACCCAAATTACC	59	GCTGGCCACAATCCGTGTAG	78	GTTCTTCTTTCTTCGTCACT
18	GATACACCAGGACCATACCT	37	TAGTACAACAACTGACAAAC	60	ATCCGTGTAGAGAATCTTCC	79	GCTTCTCGGAGTTCTTCTTT
19	GACCATACCTGTTGGATGTG	38	CAACCAAAAGTAGTACAACA	61	AGAATCTTCCTGTGAAGATA	80	TGTCTGAATAGCTTCTCGGA
20	GTTGGATGTGATATGTCCGC	39	CGTCTGGGAACAACCAAAAG	62	TGTGAAGATACTCTTGTTAA	81	TATCCAGCATTGTCTGAATA
21	ATATGTCCGCACCAAGAACA	40	ACAGCGAGTACGTCTGGGAA	63	CTCTTGTTAAACAACCAGCA	82	GGTCCAGGTGTATCCAGCAT
22	ACCAAGAACATGTGTTACCG	41	CAAAACAACAACAGCGAGTA	64	ACAACCAGCATCTTGGGATG	83	CAACAGGTACGGTCCAGGTG
23	TGTGTTACCGATGATCCCAA	42	AAAAAGGAAACAAAACAACA	65	TCTTGGGATGGTCATGCAAT	84	AGATGACATCCAACAGGTAC
43	ATGATCCCAAGTGGTGGCACT	44	AGTGCCACCACTTGGGATCAT	85	GTCATGCAATGGGAAGATCGG	86	CCGATCTTCCCATTGCATGAC
45	ATGATCCCAAATGGTGGCACT	46	AGTGCCACCATTTGGGATCAT	87	GTCATGCAATTGGAAGATCGG	88	CCGATCTTCCAATTGCATGAC

All oligonucleotides noted as being in the forward direction are located on the 5′ side of either PM1 or PM2 mutation, in reference to SEQ ID:1 and SEQ ID:2. All oligonucleotides noted as being in the reverse direction are located on the 3′ side of either the PM1 or PM2 mutation, in reference to SEQ ID:3 and SEQ ID:4.

FIG. 1E

"PM1" Test

AHAS1

'Topas'

```
Ile Pro Ser Gly Gly Thr Phe Lys Asp Val      (SEQ ID NO:105)
ATC CCA AGT GGT GGC ACT TTC AAA GAT GTA      (SEQ ID NO:106)
      3' A CCA CCG TGA AAG TTT CTA C 5'    PM1 primer
                                          (SEQ ID NO:24)

         ddATP
         ddCTP        AmpliTaq®
         ddGTP        DNA polymerase
         ddTTP

Ile Pro Ser Gly Gly Thr Phe Lys Asp Val      (SEQ ID NO:105)
ATC CCA AGT GGT GGC ACT TTC AAA GAT GTA      (SEQ ID NO:106)
     3' CA CCA CCG TGA AAG TTT CTA C 5'    PM1 primer
                                          (SEQ ID NO:107)
```

'PM1'

```
Ile Pro Asn Gly Gly Thr Phe Lys Asp Val      (SEQ ID NO:108)
ATC CCA AAT GGT GGC ACT TTC AAA GAT GTA      (SEQ ID NO:109)
      3' A CCA CCG TGA AAG TTT CTA C 5'    PM1 primer
                                          (SEQ ID NO:24)

         ddATP
         ddCTP        AmpliTaq®
         ddGTP        DNA polymerase
         ddTTP

Ile Pro Asn Gly Gly Thr Phe Lys Asp Val      (SEQ ID NO:108)
ATC CCA AAT GGT GGC ACT TTC AAA GAT GTA      (SEQ ID NO:109)
     3' TA CCA CCG TGA AAG TTT CTA C 5'    PM1 primer
                                          (SEQ ID NO:110)
```

FIG. 2

SUBSTITUTE SHEET (RULE 26)

"PM2" Test

AHAS3

'Topas'

```
Met Gln Trp Glu Asp Arg Phe Tyr Lys Ala   (SEQ ID NO :111)
ATG CAA TGG GAA GAT CGG TTC TAC AAA GCT   (SEQ ID NO :112)
        3' C CTT CTA GCC AAG ATG TTT C 5'  PM2 primer
                                           (SEQ ID NO:66)
```

ddATP
ddCTP
ddGTP
ddTTP

AmpliTaq®
DNA polymerase

```
Met Gln Trp Glu Asp Arg Phe Tyr Lys Ala   (SEQ ID NO :111)
ATG CAA TGG GAA GAT CGG TTC TAC AAA GCT   (SEQ ID NO :112)
       3' CC CTT CTA GCC AAG ATG TTT C 5'  PM2 primer
                                           (SEQ ID NO:113)
```

'PM2'

```
Met Gln Leu Glu Asp Arg Phe Tyr Lys Ala   (SEQ ID NO :114)
ATG CAA TTG GAA GAT CGG TTC TAC AAA GCT   (SEQ ID NO :115)
        3' C CTT CTA GCC AAG ATG TTT C 5'  PM2 primer
                                           (SEQ ID NO.66)
```

ddATP
ddCTP
ddGTP
ddTTP

AmpliTaq®
DNA polymerase

```
Met Gln Leu Glu Asp Arg Phe Tyr Lys Ala   (SEQ ID NO :114)
ATG CAA TTG GAA GAT CGG TTC TAC AAA GCT   (SEQ ID NO :115)
       3' AC CTT CTA GCC AAG ATG TTT C 5'  PM2 primer
                                           (SEQ ID NO.116)
```

FIG. 3

Doubled Haploid Canola Lines		
Plant number	class	GH Rating
1	"PM1/PM2"	0
2	"PM1/PM2"	0
3	"PM1/PM2"	0
4	"PM1/PM2"	0
6	"PM1/PM2"	5
7	"PM1/PM2"	5
37	"PM2"	20
38	"PM2"	20
39	"PM2"	20
40	"PM2"	25
41	"PM2"	25
42	"PM2"	25
68	"PM1"	40
69	"PM1"	40
70	"PM1"	40
71	"PM1"	40
72	"PM1"	40
73	"PM1"	45
103	WT	80
104	WT	80
105	WT	80
106	WT	80
107	WT	85
108	WT	85
-	"PM1"	-
-	"PM2"	-
-	W T	-

FIG. 4

Summary of results from "PM1" and "PM2" mutation tests with three replicates using the ABI PRISM Technology

DNA class	Plant number[2]	"PM1" mutation test Observed[3]	"PM1" mutation test Expected[4]	"PM1" mutation	"PM2" mutation test Observed[3]	"PM2" mutation test Expected[4]	"PM2" mutation
"PM1/PM2" class	1	(C)-*T*	C-*T*	yes	(C)-*A*	C-*A*	yes
	2	(C)-*T*	C-*T*	yes	(C)-*A*	C-*A*	yes
	3	(C)-*T*	C-*T*	yes	(C)-*A*	C-*A*	yes
	4	(C)-*T*	C-*T*	yes	(C)-*A*	C-*A*	yes
	6	(C)-*T*	C-*T*	yes	(C)-*A*	C-*A*	yes
	7	(C)-*T*	C-*T*	yes	(C)-*A*	C-*A*	yes
"PM2" class	37	(C)-*T*	C	yes	(C)-*A*	C-*A*	yes
	38	(C)-*T*	C	yes	C-*A*	C-*A*	yes
	39	(C)-*T*-(A)	C	yes	(C)-*A*	C-*A*	yes
	40	C	C	no	(C)-*A*	C-*A*	yes
	41	(C)-*T*	C	yes	(C)-*A*	C-*A*	yes/no
	42	(C)-*T*	C	yes	(C)-*A*	C-*A*	yes
"PM1" class	68	T	C-*T*	yes	C	C	no
	69	*T*	C-*T*	yes	C	C	no
	70	*T*	C-*T*	yes	C	C	no
	71	*T*	C-*T*	yes	C	C	no
	72	*T*	C-*T*	yes	C	C	no
	73	*T*	C-*T*	yes	C	C	no
"WT" class	103	C	C	no	C	C	no
	104	C	C	no	C	C	no
	105	C	C	no	C	C	no
	106	C	C	no	C	C	no
	107	C	C	no	C	C	no
	108	C	C	no	C	C	no
'PM1' control[1]	-	(C)-*T*	C-*T*	yes	C	C	no
'PM2' control[1]	-	C	C	no	C-*A*	C-*A*	yes
'Topas' (WT) control[1]	-	C	C	no	C	C	no

[1] 'PM1' control and 'PM2' control are DNA isolated fom "PM1" and "PM2" plants used to develop the tests.

[2] This number refers to the plant number for DH line identified in Figure 4

[3] In **Bold** and *italics* are the peaks related to the mutations and in brackets are the peaks that are not always present in all the three replicates.

[4] Expected results assuming that the AHAS amplificati on reaction using AHAS1/AHAS3 amplification primers (S ED ID NO:90 and 91) amplified similar amounts of both *AHAS1* and *AHAS3* sequences and that the PM1 ex tension primers will anneal also to the *AHAS3* sequence and the PM2 extension primers will anneal alsoto the *AHAS1* sequence.

FIG. 5

Summary of results from "PM1" and "PM2" mutation tests using the Pyrosequencing PSQ 96 Technology

	"PM1" mutation test			"PM2" mutation test		
DNA sample	Pyrosequencing results	SEQ ID NO	"PM1" Mutation	Pyrosequencing results	SEQ ID NO	"PM2" Mutation
'PM1'	CAA**A**TGGTGG	98	Yes	GGGAAGATC	99	No
'PM2'	CAAGTGGTGG	97	No	**T**GGAAGATC	100	Yes
'Topas' (WT)	CAAGTGGTGG	97	No	GGGAAGATC	99	No

FIG. 6

SEQUENCE LISTING

<110> BASF PLANT SCIENCE GmbH

<120> COMPOSITIONS AND METHODS FOR IDENTIFYING PLANTS HAVING
INCREASED TOLERANCE TO IMIDAZOLINONE HERBICIDES

<130> 15039-PCT

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                           Met Ala Ala Ala Thr Ser Ser Ser Pro
                            1               5

atc tcc tta acc gct aaa cct tct tcc aaa tcc cct cta ccc att tcc      99
Ile Ser Leu Thr Ala Lys Pro Ser Ser Lys Ser Pro Leu Pro Ile Ser
 10                  15                  20                  25

aga ttc tcc ctt ccc ttc tcc tta acc cca cag aaa gac tcc tcc cgt     147
Arg Phe Ser Leu Pro Phe Ser Leu Thr Pro Gln Lys Asp Ser Ser Arg
                 30                  35                  40

ctc cac cgt cct ctc gcc atc tcc gcc gtt ctc aac tca ccc gtc aat     195
Leu His Arg Pro Leu Ala Ile Ser Ala Val Leu Asn Ser Pro Val Asn
             45                  50                  55

gtc gca cct cct tcc cct gaa aaa acc gac aag aac aag act ttc gtc     243
Val Ala Pro Pro Ser Pro Glu Lys Thr Asp Lys Asn Lys Thr Phe Val
         60                  65                  70

tcc cgc tac gct ccc gac gag ccc cgc aag ggt gct gat atc ctc gtc     291
Ser Arg Tyr Ala Pro Asp Glu Pro Arg Lys Gly Ala Asp Ile Leu Val
     75                  80                  85
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gaa gcc ctc gag cgt caa ggc gtc gaa acc gtc ttt gct tat ccc gga 339
Glu Ala Leu Glu Arg Gln Gly Val Glu Thr Val Phe Ala Tyr Pro Gly
90 95 100 105

ggt gct tcc atg gag atc cac caa gcc ttg act cgc tcc tcc acc atc 387
Gly Ala Ser Met Glu Ile His Gln Ala Leu Thr Arg Ser Ser Thr Ile
110 115 120

cgt aac gtc ctt ccc cgt cac gaa caa gga gga gtc ttc gcc gcc gag 435
Arg Asn Val Leu Pro Arg His Glu Gln Gly Gly Val Phe Ala Ala Glu
125 130 135

ggt tac gct cgt tcc tcc ggc aaa ccg gga atc tgc ata gcc act tcg 483
Gly Tyr Ala Arg Ser Ser Gly Lys Pro Gly Ile Cys Ile Ala Thr Ser
140 145 150

ggt ccc gga gct acc aac ctc gtc agc ggg tta gca gac gcg atg ctt 531
Gly Pro Gly Ala Thr Asn Leu Val Ser Gly Leu Ala Asp Ala Met Leu
155 160 165

gac agt gtt cct ctt gtc gcc att aca gga cag gtc cct cgc cgg atg 579
Asp Ser Val Pro Leu Val Ala Ile Thr Gly Gln Val Pro Arg Arg Met
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atc ggt act gac gcc ttc caa gag aca cca atc gtt gag gta acg agg 627
Ile Gly Thr Asp Ala Phe Gln Glu Thr Pro Ile Val Glu Val Thr Arg
190 195 200

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Ser Ile Thr Lys His Asn Tyr Leu Val Met Asp Val Asp Asp Ile Pro
205 210 215

agg atc gtt caa gaa gca ttc ttt cta gct act tcc ggt aga ccc gga 723
Arg Ile Val Gln Glu Ala Phe Phe Leu Ala Thr Ser Gly Arg Pro Gly
220 225 230

ccg gtt ttg gtt gat gtt cct aag gat att cag cag cag ctt gcg att 771
Pro Val Leu Val Asp Val Pro Lys Asp Ile Gln Gln Gln Leu Ala Ile
235 240 245

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Pro Asn Trp Asp Gln Pro Met Arg Leu Pro Gly Tyr Met Ser Arg Leu
250 255 260 265

cct cag ccw ccg gaa gtt tct cag tta ggt cag atc gtt agg ttg atc 867
Pro Gln Xaa Pro Glu Val Ser Gln Leu Gly Gln Ile Val Arg Leu Ile
270 275 280

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285 290 295

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gcg agt acg ttg atg ggg ctt ggc tct tat cct tgt aac gat gag ttg  1011
Ala Ser Thr Leu Met Gly Leu Gly Ser Tyr Pro Cys Asn Asp Glu Leu
    315                 320                 325

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Ser Leu Gln Met Leu Gly Met His Gly Thr Val Tyr Ala Asn Tyr Ala
330                 335                 340                 345

gtg gag cat agt gat ttg ttg ctg gcg ttt ggt gtt agg ttt gat gac  1107
Val Glu His Ser Asp Leu Leu Leu Ala Phe Gly Val Arg Phe Asp Asp
                350                 355                 360

cgt gtc acg gga aag ctc gag gct ttc gct agc agg gct aaa att gtg  1155
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            365                 370                 375

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His Ile Asp Ile Asp Ser Ala Glu Ile Gly Lys Asn Lys Thr Pro His
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Val Ser Val Cys Gly Asp Val Lys Leu Ala Leu Gln Gly Met Asn Lys
    395                 400                 405

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Val Leu Glu Asn Arg Ala Glu Glu Leu Lys Leu Asp Phe Gly Val Trp
410                 415                 420                 425

agg agt gag ttg agc gag cag aaa cag aag ttc cct ttg agc ttc aaa  1347
Arg Ser Glu Leu Ser Glu Gln Lys Gln Lys Phe Pro Leu Ser Phe Lys
                430                 435                 440

acg ttt gga gaa gcc att cct ccg cag tac gcg att cag atc ctc gac  1395
Thr Phe Gly Glu Ala Ile Pro Pro Gln Tyr Ala Ile Gln Ile Leu Asp
            445                 450                 455

gag cta acc gaa ggg aag gca att atc agt act ggt gtt gga cag cgt  1443
Glu Leu Thr Glu Gly Lys Ala Ile Ile Ser Thr Gly Val Gly Gln Arg
        460                 465                 470

cag atg tgg gcg gcg cag ttt tac aag tac agg aag ccg aga cag tgg  1491
Gln Met Trp Ala Ala Gln Phe Tyr Lys Tyr Arg Lys Pro Arg Gln Trp
    475                 480                 485

ctg tcg tca tca ggc ctc gga gct atg ggt ttt gga ctt cct gct gcg  1539
Leu Ser Ser Ser Gly Leu Gly Ala Met Gly Phe Gly Leu Pro Ala Ala
490                 495                 500                 505
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Ile Gly Ala Ser Val Ala Asn Pro Asp Ala Ile Val Val Asp Ile Asp
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Gly Asp Gly Ser Phe Ile Met Asn Val Gln Glu Leu Ala Thr Ile Arg
            525                 530                 535

gta gag aat ctt cct gtg aag ata ctc ttg tta aac aac cag cat ctt    1683
Val Glu Asn Leu Pro Val Lys Ile Leu Leu Leu Asn Asn Gln His Leu
        540                 545                 550

ggg atg gtc atg caa tgg gaa gat cgg ttc tac aaa gct aac aga gct    1731
Gly Met Val Met Gln Trp Glu Asp Arg Phe Tyr Lys Ala Asn Arg Ala
    555                 560                 565

cac act tat ctc ggg gac ccg gca agg gag aac gag atc ttc cct aac    1779
His Thr Tyr Leu Gly Asp Pro Ala Arg Glu Asn Glu Ile Phe Pro Asn
570                 575                 580                 585

atg ctg cag ttt gca gga gct tgc ggg att cca gct gcg aga gtg acg    1827
Met Leu Gln Phe Ala Gly Ala Cys Gly Ile Pro Ala Ala Arg Val Thr
                590                 595                 600

aag aaa gaa gaa ctc cga gaa gct att cag aca atg ctg gat aca cca    1875
Lys Lys Glu Glu Leu Arg Glu Ala Ile Gln Thr Met Leu Asp Thr Pro
            605                 610                 615

gga cca tac ctg ttg gat gtg ata tgt ccg cac caa gaa cat gtg tta    1923
Gly Pro Tyr Leu Leu Asp Val Ile Cys Pro His Gln Glu His Val Leu
        620                 625                 630

ccg atg atc cca aat ggt ggc act ttc aaa gat gta ata aca gaa ggg    1971
Pro Met Ile Pro Asn Gly Gly Thr Phe Lys Asp Val Ile Thr Glu Gly
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Thr Ser Ser Ser Pro Ile Ser Leu Thr Ala Lys Pro Ser Ser Lys Ser
5 10 15 20

cct cta ccc att tcc aga ttc tcc ctt ccc ttc tcc tta acc cca cag 150
Pro Leu Pro Ile Ser Arg Phe Ser Leu Pro Phe Ser Leu Thr Pro Gln
25 30 35

aaa ccc tcc tcc cgt ctc cac cgt cca ctc gcc atc tcc gcc gtt ctc 198
Lys Pro Ser Ser Arg Leu His Arg Pro Leu Ala Ile Ser Ala Val Leu
40 45 50

aac tca ccc gtc aat gtc gca cct gaa aaa acc gac aag atc aag act 246
Asn Ser Pro Val Asn Val Ala Pro Glu Lys Thr Asp Lys Ile Lys Thr
55 60 65

ttc atc tcc cgc tac gct ccc gac gag ccc cgc aag ggt gct gat atc 294
Phe Ile Ser Arg Tyr Ala Pro Asp Glu Pro Arg Lys Gly Ala Asp Ile
70 75 80

ctc gtg gaa gcc ctc gag cgt caa ggc gtc gaa acc gtc ttc gct tat 342
Leu Val Glu Ala Leu Glu Arg Gln Gly Val Glu Thr Val Phe Ala Tyr
85 90 95 100

ccc gga ggt gcc tcc atg gag atc cac caa gcc ttg act cgc tcc tcc 390
Pro Gly Gly Ala Ser Met Glu Ile His Gln Ala Leu Thr Arg Ser Ser
105 110 115

acc atc cgt aac gtc ctc ccc cgt cac gaa caa gga gga gtc ttc gcc 438
Thr Ile Arg Asn Val Leu Pro Arg His Glu Gln Gly Gly Val Phe Ala
120 125 130

gcc gag ggt tac gct cgt tcc tcc ggc aaa ccg gga atc tgc ata gcc 486
Ala Glu Gly Tyr Ala Arg Ser Ser Gly Lys Pro Gly Ile Cys Ile Ala
135 140 145

act tcg ggt ccc gga gct acc aac ctc gtc agc ggg tta gcc gac gcg 534
Thr Ser Gly Pro Gly Ala Thr Asn Leu Val Ser Gly Leu Ala Asp Ala
150 155 160

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atg ctt gac agt gtt cct ctc gtc gcc atc aca gga cag gtc cct cgc   582
Met Leu Asp Ser Val Pro Leu Val Ala Ile Thr Gly Gln Val Pro Arg
165                 170                 175                 180

cgg atg atc ggt act gac gcg ttc caa gag acg cca atc gtt gag gta   630
Arg Met Ile Gly Thr Asp Ala Phe Gln Glu Thr Pro Ile Val Glu Val
                185                 190                 195

acg agg tct att acg aaa cat aac tat ctg gtg atg gat gtt gat gac   678
Thr Arg Ser Ile Thr Lys His Asn Tyr Leu Val Met Asp Val Asp Asp
            200                 205                 210

ata cct agg atc gtt caa gaa gca ttc ttt cta gct act tcc ggt aga   726
Ile Pro Arg Ile Val Gln Glu Ala Phe Phe Leu Ala Thr Ser Gly Arg
        215                 220                 225

ccc gga ccg gtt ttg gtt gat gtt cct aag gat att cag cag cag ctt   774
Pro Gly Pro Val Leu Val Asp Val Pro Lys Asp Ile Gln Gln Gln Leu
    230                 235                 240

gcg att cct aac tgg gat caa cct atg cgc ttg cct ggc tac atg tct   822
Ala Ile Pro Asn Trp Asp Gln Pro Met Arg Leu Pro Gly Tyr Met Ser
245                 250                 255                 260

agg ctg cct cag cca ccg gaa gtt tct cag tta ggc cag atc gtt agg   870
Arg Leu Pro Gln Pro Pro Glu Val Ser Gln Leu Gly Gln Ile Val Arg
                265                 270                 275

ttg atc tcg gag tct aag agg cct gtt ttg tac gtt ggt ggt gga agc   918
Leu Ile Ser Glu Ser Lys Arg Pro Val Leu Tyr Val Gly Gly Gly Ser
            280                 285                 290

ttg aac tcg agt gaa gaa ctg ggg aga ttt gtc gag ctt act ggg atc   966
Leu Asn Ser Ser Glu Glu Leu Gly Arg Phe Val Glu Leu Thr Gly Ile
        295                 300                 305

cct gtt gcg agt acg ctg atg ggg ctt ggc tct tat cct tgt aac gat   1014
Pro Val Ala Ser Thr Leu Met Gly Leu Gly Ser Tyr Pro Cys Asn Asp
    310                 315                 320

gag ttg tcc ctg cag atg ctt ggc atg cac ggg act gtg tat gct aac   1062
Glu Leu Ser Leu Gln Met Leu Gly Met His Gly Thr Val Tyr Ala Asn
325                 330                 335                 340

tac gct gtg gag cat agt gat ttg ttg ctg gcg ttt ggt gtt agg ttt   1110
Tyr Ala Val Glu His Ser Asp Leu Leu Leu Ala Phe Gly Val Arg Phe
                345                 350                 355

gat gac cgt gtc acg gga aag ctc gag gcg ttt gcg agc agg gct aag   1158
Asp Asp Arg Val Thr Gly Lys Leu Glu Ala Phe Ala Ser Arg Ala Lys
            360                 365                 370
```

```
att gtg cac ata gac att gat tct gct gag att ggg aag aat aag aca     1206
Ile Val His Ile Asp Ile Asp Ser Ala Glu Ile Gly Lys Asn Lys Thr
        375                 380                 385

cct cac gtg tct gtg tgt ggt gat gta aag ctg gct ttg caa ggg atg     1254
Pro His Val Ser Val Cys Gly Asp Val Lys Leu Ala Leu Gln Gly Met
    390                 395                 400

aac aag gtt ctt gag aac cgg gcg gag gag ctc aag ctt gat ttc ggt     1302
Asn Lys Val Leu Glu Asn Arg Ala Glu Glu Leu Lys Leu Asp Phe Gly
405                 410                 415                 420

gtt tgg agg agt gag ttg agc gag cag aaa cag aag ttc ccg ttg agc     1350
Val Trp Arg Ser Glu Leu Ser Glu Gln Lys Gln Lys Phe Pro Leu Ser
                425                 430                 435

ttc aaa acg ttt gga gaa gcc att cct ccg cag tac gcg att cag gtc     1398
Phe Lys Thr Phe Gly Glu Ala Ile Pro Pro Gln Tyr Ala Ile Gln Val
            440                 445                 450

cta gac gag cta acc caa ggg aag gca att atc agn act ggt gtt gga     1446
Leu Asp Glu Leu Thr Gln Gly Lys Ala Ile Ile Xaa Thr Gly Val Gly
        455                 460                 465

cag cat cag atg tgg gcg gcg cag ttt tac aag tac agg aag ccg agg     1494
Gln His Gln Met Trp Ala Ala Gln Phe Tyr Lys Tyr Arg Lys Pro Arg
    470                 475                 480

cag tgg ctg tcg tcc tca gga ctc gga gct atg ggt ttc gga ctt cct     1542
Gln Trp Leu Ser Ser Ser Gly Leu Gly Ala Met Gly Phe Gly Leu Pro
485                 490                 495                 500

gct gcg att gga gcg tct gtg gcg aac cct gat gcg att gtt gtg gac     1590
Ala Ala Ile Gly Ala Ser Val Ala Asn Pro Asp Ala Ile Val Val Asp
                505                 510                 515

att gac ggt gat gga agc ttc ata atg aac gtt caa gag ctg gcc aca     1638
Ile Asp Gly Asp Gly Ser Phe Ile Met Asn Val Gln Glu Leu Ala Thr
            520                 525                 530

atc cgt gta gag aat ctt cct gtg aag ata ctc ttg tta aac aac cag     1686
Ile Arg Val Glu Asn Leu Pro Val Lys Ile Leu Leu Leu Asn Asn Gln
        535                 540                 545

cat ctt ggg atg gtc atg caa ttg gaa gat cgg ttc tac aaa gct aac     1734
His Leu Gly Met Val Met Gln Leu Glu Asp Arg Phe Tyr Lys Ala Asn
    550                 555                 560

aga gct cac act tat ctc ggg gac ccg gca agg gag aac gag atc ttc     1782
Arg Ala His Thr Tyr Leu Gly Asp Pro Ala Arg Glu Asn Glu Ile Phe
565                 570                 575                 580
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cct aac atg ctg cag ttt gca gga gct tgc ggg att cca gct gcg aga    1830
Pro Asn Met Leu Gln Phe Ala Gly Ala Cys Gly Ile Pro Ala Ala Arg
                585                 590                 595

gtg acg aag aaa gaa gaa ctc cga gaa gct att cag aca atg ctg gat    1878
Val Thr Lys Lys Glu Glu Leu Arg Glu Ala Ile Gln Thr Met Leu Asp
            600                 605                 610

aca cct gga ccg tac ctg ttg gat gcc atc tgt ccg cac caa gaa cat    1926
Thr Pro Gly Pro Tyr Leu Leu Asp Ala Ile Cys Pro His Gln Glu His
        615                 620                 625

gtg tta ccg atg atc cca agt ggt ggc act ttc aaa gat gta ata acc    1974
Val Leu Pro Met Ile Pro Ser Gly Gly Thr Phe Lys Asp Val Ile Thr
    630                 635                 640

gaa ggg gat ggt cgc act aag tac tgagagatga agctggtgat ccatcgtatg  2028
Glu Gly Asp Gly Arg Thr Lys Tyr
645                 650

gtaaaagact tagtttcagt tttcagtttc ttttgtgtgg taatttgggt ttgtcagttg 2088

ttgttytgct tttggtttgt tcccnkac                                     2116


<210> 3
<211> 2083
<212> DNA
<213> Brassica napus

<220>
<221> CDS
<222> (25)..(1989)

<400> 3
tcatcatctc tctctcctct aacc atg gcg gcg gca aca tcg tct tct ccg      51
                           Met Ala Ala Ala Thr Ser Ser Ser Pro
                           1               5

atc tcc tta acc gct aaa cct tct tcc aaa tcc cct cta ccc att tcc      99
Ile Ser Leu Thr Ala Lys Pro Ser Ser Lys Ser Pro Leu Pro Ile Ser
10                  15                  20                  25

aga ttc tcc ctt ccc ttc tcc tta acc cca cag aaa gac tcc tcc cgt     147
Arg Phe Ser Leu Pro Phe Ser Leu Thr Pro Gln Lys Asp Ser Ser Arg
                30                  35                  40

ctc cac cgt cct ctc gcc atc tcc gcc gtt ctc aac tca ccc gtc aat     195
Leu His Arg Pro Leu Ala Ile Ser Ala Val Leu Asn Ser Pro Val Asn
            45                  50                  55

gtc gca cct cct tcc cct gaa aaa acc gac aag aac aag act ttc gtc     243
Val Ala Pro Pro Ser Pro Glu Lys Thr Asp Lys Asn Lys Thr Phe Val
        60                  65                  70
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tcc cgc tac gct ccc gac gag ccc cgc aag ggt gct gat atc ctc gtc   291
Ser Arg Tyr Ala Pro Asp Glu Pro Arg Lys Gly Ala Asp Ile Leu Val
     75                  80                  85

gaa gcc ctc gag cgt caa ggc gtc gaa acc gtc ttt gct tat ccc gga   339
Glu Ala Leu Glu Arg Gln Gly Val Glu Thr Val Phe Ala Tyr Pro Gly
 90                  95                 100                 105

ggt gct tcc atg gag atc cac caa gcc ttg act cgc tcc tcc acc atc   387
Gly Ala Ser Met Glu Ile His Gln Ala Leu Thr Arg Ser Ser Thr Ile
                110                 115                 120

cgt aac gtc ctt ccc cgt cac gaa caa gga gga gtc ttc gcc gcc gag   435
Arg Asn Val Leu Pro Arg His Glu Gln Gly Gly Val Phe Ala Ala Glu
            125                 130                 135

ggt tac gct cgt tcc tcc ggc aaa ccg gga atc tgc ata gcc act tcg   483
Gly Tyr Ala Arg Ser Ser Gly Lys Pro Gly Ile Cys Ile Ala Thr Ser
        140                 145                 150

ggt ccc gga gct acc aac ctc gtc agc ggg tta gca gac gcg atg ctt   531
Gly Pro Gly Ala Thr Asn Leu Val Ser Gly Leu Ala Asp Ala Met Leu
    155                 160                 165

gac agt gtt cct ctt gtc gcc att aca gga cag gtc cct cgc cgg atg   579
Asp Ser Val Pro Leu Val Ala Ile Thr Gly Gln Val Pro Arg Arg Met
170                 175                 180                 185

atc ggt act gac gcc ttc caa gag aca cca atc gtt gag gta acg agg   627
Ile Gly Thr Asp Ala Phe Gln Glu Thr Pro Ile Val Glu Val Thr Arg
                190                 195                 200

tct att acg aaa cat aac tat ttg gtg atg gat gtt gat gac ata cct   675
Ser Ile Thr Lys His Asn Tyr Leu Val Met Asp Val Asp Asp Ile Pro
            205                 210                 215

agg atc gtt caa gaa gct ttc ttt cta gct act tcc ggt aga ccc gga   723
Arg Ile Val Gln Glu Ala Phe Phe Leu Ala Thr Ser Gly Arg Pro Gly
        220                 225                 230

ccg gtt ttg gtt gat gtt cct aag gat att cag cag cag ctt gcg att   771
Pro Val Leu Val Asp Val Pro Lys Asp Ile Gln Gln Gln Leu Ala Ile
    235                 240                 245

cct aac tgg gat caa cct atg cgc tta cct ggc tac atg tct agg ttg   819
Pro Asn Trp Asp Gln Pro Met Arg Leu Pro Gly Tyr Met Ser Arg Leu
250                 255                 260                 265

cct cag cct ccg gaa gtt tct cag tta ggt cag atc gtt agg ttg atc   867
Pro Gln Pro Pro Glu Val Ser Gln Leu Gly Gln Ile Val Arg Leu Ile
                270                 275                 280
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tcg gag tct aag agg cct gtt ttg tac gtt ggt ggt gga agc ttg aac  915
Ser Glu Ser Lys Arg Pro Val Leu Tyr Val Gly Gly Gly Ser Leu Asn
            285                 290                 295

tcg agt gaa gaa ctg ggg aga ttt gtc gag ctt act ggg atc ccc gtt  963
Ser Ser Glu Glu Leu Gly Arg Phe Val Glu Leu Thr Gly Ile Pro Val
        300                 305                 310

gcg agt act ttg atg ggg ctt ggc tct tat cct tgt aac gat gag ttg  1011
Ala Ser Thr Leu Met Gly Leu Gly Ser Tyr Pro Cys Asn Asp Glu Leu
    315                 320                 325

tcc ctg cag atg ctt ggc atg cac ggg act gtg tat gct aac tac gct  1059
Ser Leu Gln Met Leu Gly Met His Gly Thr Val Tyr Ala Asn Tyr Ala
330                 335                 340                 345

gtg gag cat agt gat ttg ttg ctg gcg ttt ggt gtt agg ttt gat gac  1107
Val Glu His Ser Asp Leu Leu Leu Ala Phe Gly Val Arg Phe Asp Asp
                350                 355                 360

cgt gtc acg gga aag ctc gag gct ttc gct agc agg gct aaa att gtg  1155
Arg Val Thr Gly Lys Leu Glu Ala Phe Ala Ser Arg Ala Lys Ile Val
            365                 370                 375

cac ata gac att gat tct gct gag att ggg aag aat aag aca cct cac  1203
His Ile Asp Ile Asp Ser Ala Glu Ile Gly Lys Asn Lys Thr Pro His
        380                 385                 390

gtg tct gtg tgt ggt gat gta aag ctg gct ttg caa ggg atg aac aag  1251
Val Ser Val Cys Gly Asp Val Lys Leu Ala Leu Gln Gly Met Asn Lys
    395                 400                 405

gtt ctt gag aac cgg gcg gag gag ctc aag ctt gat ttc ggt gtt tgg  1299
Val Leu Glu Asn Arg Ala Glu Glu Leu Lys Leu Asp Phe Gly Val Trp
410                 415                 420                 425

agg agt gag ttg agc gag cag aaa cag aag ttc cct ttg agc ttc aaa  1347
Arg Ser Glu Leu Ser Glu Gln Lys Gln Lys Phe Pro Leu Ser Phe Lys
                430                 435                 440

acg ttt gga gaa gcc att cct ccg cag tac gcg att cag atc ctc gac  1395
Thr Phe Gly Glu Ala Ile Pro Pro Gln Tyr Ala Ile Gln Ile Leu Asp
            445                 450                 455

gag cta acc gaa ggg aag gca att atc agt act ggt gtt gga cag cat  1443
Glu Leu Thr Glu Gly Lys Ala Ile Ile Ser Thr Gly Val Gly Gln His
        460                 465                 470

cag atg tgg gcg gcg cag ttt tac aag tac agg aag ccg aga cag tgg  1491
Gln Met Trp Ala Ala Gln Phe Tyr Lys Tyr Arg Lys Pro Arg Gln Trp
    475                 480                 485
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ctg tcg tca tca ggc ctc gga gct atg ggt ttt gga ctt cct gct gcg   1539
Leu Ser Ser Ser Gly Leu Gly Ala Met Gly Phe Gly Leu Pro Ala Ala
490                 495                 500                 505

att gga gcg tct gtg gcg aac cct gat gcg att gtt gtg gat att gac   1587
Ile Gly Ala Ser Val Ala Asn Pro Asp Ala Ile Val Val Asp Ile Asp
                510                 515                 520

ggt gat gga agc ttc ata atg aac gtt caa gag ctg gcc aca atc cgt   1635
Gly Asp Gly Ser Phe Ile Met Asn Val Gln Glu Leu Ala Thr Ile Arg
            525                 530                 535

gta gag aat ctt cct gtg aag ata ctc ttg tta aac aac cag cat ctt   1683
Val Glu Asn Leu Pro Val Lys Ile Leu Leu Leu Asn Asn Gln His Leu
        540                 545                 550

ggg atg gtc atg caa tgg gaa gat cgg ttc tac aaa gct aac aga gct   1731
Gly Met Val Met Gln Trp Glu Asp Arg Phe Tyr Lys Ala Asn Arg Ala
    555                 560                 565

cac act tat ctc ggg gac ccg gca agg gag aac gag atc ttc cct aac   1779
His Thr Tyr Leu Gly Asp Pro Ala Arg Glu Asn Glu Ile Phe Pro Asn
570                 575                 580                 585

atg ctg cag ttt gca gga gct tgc ggg att cca gct gcg aga gtg acg   1827
Met Leu Gln Phe Ala Gly Ala Cys Gly Ile Pro Ala Ala Arg Val Thr
                590                 595                 600

aag aaa gaa gaa ctc cga gaa gct att cag aca atg ctg gat aca cca   1875
Lys Lys Glu Glu Leu Arg Glu Ala Ile Gln Thr Met Leu Asp Thr Pro
            605                 610                 615

gga cca tac ctg ttg gat gtg ata tgt ccg cac caa gaa cat gtg tta   1923
Gly Pro Tyr Leu Leu Asp Val Ile Cys Pro His Gln Glu His Val Leu
        620                 625                 630

ccg atg atc cca agt ggt ggc act ttc aaa gat gta ata aca gaa ggg   1971
Pro Met Ile Pro Ser Gly Gly Thr Phe Lys Asp Val Ile Thr Glu Gly
    635                 640                 645

gat ggt cgc act aag tac tgagagatga agctggtgat cgatcatatg          2019
Asp Gly Arg Thr Lys Tyr
650                 655

gtaaaagact tagtttcagt ttccagtttc ttttgtgtgg taatttgggt ttgtcagttg 2079

ttgt                                                              2083
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<210> 4
<211> 2116
<212> DNA
<213> Brassica napus

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<220>
<221> CDS
<222> (43)..(1998)

<220>
<221> modified_base
<222> (21)..(21)
<223> a, c, g, t, other or unknown

<400> 4
ttmmacatct ctctctcatt ncactctctc cctcatctaa cc atg gcg gcg gca      54
                                              Met Ala Ala Ala
                                              1

aca tcg cct tct ccg atc tcc tta acc gct aaa cct tct tcc aaa tcc     102
Thr Ser Pro Ser Pro Ile Ser Leu Thr Ala Lys Pro Ser Ser Lys Ser
5                   10                  15                  20

cct cta ccc att tcc aga ttc tcc ctt ccc ttc tcc tta acc cca cag     150
Pro Leu Pro Ile Ser Arg Phe Ser Leu Pro Phe Ser Leu Thr Pro Gln
                25                  30                  35

aaa ccc tcc tcc cgt ctc cac cgt cca ctc gcc atc tcc gcc gtt ctc     198
Lys Pro Ser Ser Arg Leu His Arg Pro Leu Ala Ile Ser Ala Val Leu
            40                  45                  50

aac tca ccc gtc aat gtc gca cct gaa aaa acc gac aag atc aag act     246
Asn Ser Pro Val Asn Val Ala Pro Glu Lys Thr Asp Lys Ile Lys Thr
        55                  60                  65

ttc atc tcc cgc tac gct ccc gac gag ccc cgc aag ggt gct gat atc     294
Phe Ile Ser Arg Tyr Ala Pro Asp Glu Pro Arg Lys Gly Ala Asp Ile
    70                  75                  80

ctc gtg gaa gcc ctc gag cgt caa ggc gtc gaa acc gtc ttc gct tat     342
Leu Val Glu Ala Leu Glu Arg Gln Gly Val Glu Thr Val Phe Ala Tyr
85                  90                  95                  100

ccc gga ggt gcc tcc atg gag atc cac caa gcc ttg act cgc tcc tcc     390
Pro Gly Gly Ala Ser Met Glu Ile His Gln Ala Leu Thr Arg Ser Ser
                105                 110                 115

acc atc cgt aac gtc ctc ccc cgt cac gaa caa gga gga gtc ttc gcc     438
Thr Ile Arg Asn Val Leu Pro Arg His Glu Gln Gly Gly Val Phe Ala
            120                 125                 130

gcc gag ggt tac gct cgt tcc tcc ggc aaa ccg gga atc tgc ata gcc     486
Ala Glu Gly Tyr Ala Arg Ser Ser Gly Lys Pro Gly Ile Cys Ile Ala
        135                 140                 145

act tcg ggt ccc gga gct acc aac ctc gtc agc ggg tta gcc gac gcg     534
Thr Ser Gly Pro Gly Ala Thr Asn Leu Val Ser Gly Leu Ala Asp Ala
    150                 155                 160
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atg ctt gac agt gtt cct ctc gtc gcc atc aca gga cag gtc cct cgc  582
Met Leu Asp Ser Val Pro Leu Val Ala Ile Thr Gly Gln Val Pro Arg
165                 170                 175                 180

cgg atg atc ggt act gac gcg ttc caa gag acg cca atc gtt gag gta  630
Arg Met Ile Gly Thr Asp Ala Phe Gln Glu Thr Pro Ile Val Glu Val
                185                 190                 195

acg agg tct att acg aaa cat aac tat ctg gtg atg gat gtt gat gac  678
Thr Arg Ser Ile Thr Lys His Asn Tyr Leu Val Met Asp Val Asp Asp
            200                 205                 210

ata cct agg atc gtt caa gaa gca ttc ttt cta gct act tcc ggt aga  726
Ile Pro Arg Ile Val Gln Glu Ala Phe Phe Leu Ala Thr Ser Gly Arg
        215                 220                 225

ccc gga ccg gtt ttg gtt gat gtt cct aag gat att cag cag cag ctt  774
Pro Gly Pro Val Leu Val Asp Val Pro Lys Asp Ile Gln Gln Gln Leu
    230                 235                 240

gcg att cct aac tgg gat caa cct atg cgc ttg cct ggc tac atg tct  822
Ala Ile Pro Asn Trp Asp Gln Pro Met Arg Leu Pro Gly Tyr Met Ser
245                 250                 255                 260

agg ctg cct cag cca ccg gaa gtt tct cag tta ggc cag atc gtt agg  870
Arg Leu Pro Gln Pro Pro Glu Val Ser Gln Leu Gly Gln Ile Val Arg
                265                 270                 275

ttg atc tcg gag tct aag agg cct gtt ttg tac gtt ggt ggt gga agc  918
Leu Ile Ser Glu Ser Lys Arg Pro Val Leu Tyr Val Gly Gly Gly Ser
            280                 285                 290

ttg aac tcg agt gag gaa ctg ggg aga ttt gtc gag ctt act ggg atc  966
Leu Asn Ser Ser Glu Glu Leu Gly Arg Phe Val Glu Leu Thr Gly Ile
        295                 300                 305

cct gtt gcg agt acg ttg atg ggg ctt ggc tct tat cct tgt aac gat  1014
Pro Val Ala Ser Thr Leu Met Gly Leu Gly Ser Tyr Pro Cys Asn Asp
    310                 315                 320

gag ttg tcc ctg cag atg ctt ggc atg cac ggg act gtg tat gct aac  1062
Glu Leu Ser Leu Gln Met Leu Gly Met His Gly Thr Val Tyr Ala Asn
325                 330                 335                 340

tac gct gtg gag cat agt gat ttg ttg ctg gcg ttt ggt gtt agg ttt  1110
Tyr Ala Val Glu His Ser Asp Leu Leu Leu Ala Phe Gly Val Arg Phe
                345                 350                 355

gat gac cgt gtc acg gga aag ctc gag gcg ttt gcg agc agg gct aag  1158
Asp Asp Arg Val Thr Gly Lys Leu Glu Ala Phe Ala Ser Arg Ala Lys
            360                 365                 370
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att gtg cac ata gac att gat tct gct gag att ggg aag aat aag aca 1206
Ile Val His Ile Asp Ile Asp Ser Ala Glu Ile Gly Lys Asn Lys Thr
375 380 385

cct cac gtg tct gtg tgt ggt gat gta aag ctg gct ttg caa ggg atg 1254
Pro His Val Ser Val Cys Gly Asp Val Lys Leu Ala Leu Gln Gly Met
390 395 400

aac aag gtt ctt gag aac cgg gcg gag gag ctc aag ctt gat ttc ggt 1302
Asn Lys Val Leu Glu Asn Arg Ala Glu Glu Leu Lys Leu Asp Phe Gly
405 410 415 420

gtt tgg agg agt gag ttg agc gag cag aaa cag aag ttc ccg ttg agc 1350
Val Trp Arg Ser Glu Leu Ser Glu Gln Lys Gln Lys Phe Pro Leu Ser
425 430 435

ttc aaa acg ttt gga gaa gcc att cct ccg cag tac gcg att cag gtc 1398
Phe Lys Thr Phe Gly Glu Ala Ile Pro Pro Gln Tyr Ala Ile Gln Val
440 445 450

cta gac gag cta acc caa ggg aag gca att atc agt act ggt gtt gga 1446
Leu Asp Glu Leu Thr Gln Gly Lys Ala Ile Ile Ser Thr Gly Val Gly
455 460 465

cag cat cag atg tgg gcg gcg cag ttt tac aag tac agg aag ccg agg 1494
Gln His Gln Met Trp Ala Ala Gln Phe Tyr Lys Tyr Arg Lys Pro Arg
470 475 480

cag tgg ctg tcg tcc tca gga ctc gga gct atg ggt ttc gga ctt cct 1542
Gln Trp Leu Ser Ser Ser Gly Leu Gly Ala Met Gly Phe Gly Leu Pro
485 490 495 500

gct gcg att gga gcg tct gtg gcg aac cct gat gcg att gtt gtg gac 1590
Ala Ala Ile Gly Ala Ser Val Ala Asn Pro Asp Ala Ile Val Val Asp
505 510 515

att gac ggt gat gga agc ttc ata atg aac gtt caa gag ctg gcc aca 1638
Ile Asp Gly Asp Gly Ser Phe Ile Met Asn Val Gln Glu Leu Ala Thr
520 525 530

atc cgt gta gag aat ctt cct gtg aag ata ctc ttg tta aac aac cag 1686
Ile Arg Val Glu Asn Leu Pro Val Lys Ile Leu Leu Leu Asn Asn Gln
535 540 545

cat ctt ggg atg gtc atg caa tgg gaa gat cgg ttc tac aaa gct aac 1734
His Leu Gly Met Val Met Gln Trp Glu Asp Arg Phe Tyr Lys Ala Asn
550 555 560

aga gct cac act tat ctc ggg gac ccg gca agg gag aac gag atc ttc 1782
Arg Ala His Thr Tyr Leu Gly Asp Pro Ala Arg Glu Asn Glu Ile Phe
565 570 575 580

```
cct aac atg ctg cag ttt gca gga gct tgc ggg att cca gct gcg aga   1830
Pro Asn Met Leu Gln Phe Ala Gly Ala Cys Gly Ile Pro Ala Ala Arg
                585                 590                 595

gtg acg aag aaa gaa gaa ctc cga gaa gct att cag aca atg ctg gat   1878
Val Thr Lys Lys Glu Glu Leu Arg Glu Ala Ile Gln Thr Met Leu Asp
            600                 605                 610

aca cct gga ccg tac ctg ttg gat gtc atc tgt ccg cac caa gaa cat   1926
Thr Pro Gly Pro Tyr Leu Leu Asp Val Ile Cys Pro His Gln Glu His
        615                 620                 625

gtg tta ccg atg atc cca agt ggt ggc act ttc gaa gat gta ata acc   1974
Val Leu Pro Met Ile Pro Ser Gly Gly Thr Phe Glu Asp Val Ile Thr
    630                 635                 640

gaa ggg gat ggt cgc act aag tac tgagagatga agctggtgat ccatcatatg  2028
Glu Gly Asp Gly Arg Thr Lys Tyr
645                 650

gtaaaagact tagtttcagt ttacagtttc ttttgtgtgg taatttgggt ttgtcagttg 2088

ttgttctgct tttggtttgt tccccwkac                                   2116
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<210> 5
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 5
ttatctcggg gacccggcaa 20

<210> 6
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 6
gacccggcaa gggagaacga 20

<210> 7
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 7
gggagaacga gatcttccct 20

<210> 8
<211> 20
<212> DNA
<213> Artificial Sequence

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<210> 9
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<400> 9
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<210> 10
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<400> 10
agtttgcagg agcttgcggg 20

<210> 11
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<210> 13
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<210> 14
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<223> Description of Artificial Sequence: Synthetic
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gaagaaagaa gaactccgag 20

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<210> 16
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<210> 17
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<210> 18
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<223> Description of Artificial Sequence: Synthetic
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<400> 18
gatacaccag gaccatacct 20

<210> 19
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<210> 20
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<210> 21
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<210> 22
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<210> 23
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<210> 24
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<210> 26
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<210> 27
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<400> 27
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<210> 28
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<210> 29
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<400> 29
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<210> 30
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<400> 30
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<210> 31
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<400> 31
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<210> 32
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<210> 33
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<400> 33
aactggaaac tgaaactaag 20

<210> 34
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oligonucleotide

<400> 34
acacaaaaga aactggaaac 20

<210> 35
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<210> 36
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<400> 36
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<210> 37
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<400> 37
tagtacaaca actgacaaac 20

<210> 38
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<400> 38
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<210> 39
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<400> 39
cgtctgggaa caaccaaaag 20

<210> 40
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<400> 40
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<210> 41
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<400> 41
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<210> 42
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<400> 42
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<400> 43
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<210> 44
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<400> 44
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<210> 45
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<400> 45
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<210> 46
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<400> 46
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<210> 47
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<400> 47
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<210> 48
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<210> 49
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<400> 49
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<210> 50
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<400> 50
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<210> 51
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<210> 52
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<400> 52
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<210> 53
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<210> 54
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<210> 55
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<210> 56
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<400> 56
tgatggaagc ttcataatga 20

<210> 57
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<400> 57
ttcataatga acgttcaaga 20

<210> 58
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<400> 58
acgttcaaga gctggccaca 20

<210> 59
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<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 59
gctggccaca atccgtgtag 20

<210> 60
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<220>
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<400> 60
atccgtgtag agaatcttcc 20

<210> 61
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<220>
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<400> 61
agaatcttcc tgtgaagata 20

<210> 62
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<400> 62
tgtgaagata ctcttgttaa 20

<210> 63
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<400> 63
ctcttgttaa acaaccagca 20

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<400> 64
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<210> 66
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<210> 68
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<210> 70
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<210> 75
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gaatcccgca agctcctgca 20

<210> 76
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<210> 78
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<210> 79
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<210> 80
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<210> 81
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tatccagcat tgtctgaata 20

<210> 82
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<400> 82
ggtccaggtg tatccagcat 20

<210> 83
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oligonucleotide

<400> 83
caacaggtac ggtccaggtg 20

<210> 84
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<400> 84
agatgacatc caacaggtac 20

<210> 85
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<400> 85
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<210> 86
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<210> 87
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<210> 88
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<400> 88
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<210> 89
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<400> 89
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<210> 90
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<400> 90
ggcgtttggt gttaggtttg a 21

<210> 91
<211> 21
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<220>
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primer

<400> 91
cgtctgggaa caaccaaaag t 21

<210> 92
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<220>
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primer

<400> 92
ggaaagctcg aggctttcgc t 21

<210> 93
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<220>
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<400> 93
atcaccagct tcatctctca gt 22

<210> 94
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<220>
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<400> 94
ggaaagctcg aggcgtttgc g 21

<210> 95
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<220>
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<400> 95
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<210> 96
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gggatggtca tgcaat 16

<210> 97
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<210> 98
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<400> 98
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<210> 99
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primer

<400> 99
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<210> 100
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<210> 101
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Ser Ser Lys Ser Pro Leu Pro Ile Ser Arg Phe Ser Leu Pro Phe Ser
20 25 30

Leu Thr Pro Gln Lys Asp Ser Ser Arg Leu His Arg Pro Leu Ala Ile
35 40 45

Ser Ala Val Leu Asn Ser Pro Val Asn Val Ala Pro Pro Ser Pro Glu
50 55 60

Lys Thr Asp Lys Asn Lys Thr Phe Val Ser Arg Tyr Ala Pro Asp Glu
65 70 75 80

Pro Arg Lys Gly Ala Asp Ile Leu Val Glu Ala Leu Glu Arg Gln Gly
85 90 95

Val Glu Thr Val Phe Ala Tyr Pro Gly Gly Ala Ser Met Glu Ile His
100 105 110

Gln Ala Leu Thr Arg Ser Ser Thr Ile Arg Asn Val Leu Pro Arg His
115 120 125

Glu Gln Gly Gly Val Phe Ala Ala Glu Gly Tyr Ala Arg Ser Ser Gly
130 135 140

Lys Pro Gly Ile Cys Ile Ala Thr Ser Gly Pro Gly Ala Thr Asn Leu
145 150 155 160

Val Ser Gly Leu Ala Asp Ala Met Leu Asp Ser Val Pro Leu Val Ala
165 170 175

Ile Thr Gly Gln Val Pro Arg Arg Met Ile Gly Thr Asp Ala Phe Gln
180 185 190

Glu Thr Pro Ile Val Glu Val Thr Arg Ser Ile Thr Lys His Asn Tyr
195 200 205

Leu Val Met Asp Val Asp Asp Ile Pro Arg Ile Val Gln Glu Ala Phe
210 215 220

Phe Leu Ala Thr Ser Gly Arg Pro Gly Pro Val Leu Val Asp Val Pro
225 230 235 240

Lys Asp Ile Gln Gln Gln Leu Ala Ile Pro Asn Trp Asp Gln Pro Met
245 250 255

Arg Leu Pro Gly Tyr Met Ser Arg Leu Pro Gln Xaa Pro Glu Val Ser
260 265 270

Gln Leu Gly Gln Ile Val Arg Leu Ile Ser Glu Ser Lys Arg Pro Val
275 280 285

Leu Tyr Val Gly Gly Gly Ser Leu Asn Ser Ser Glu Glu Leu Gly Arg
290 295 300

Phe Val Glu Leu Thr Gly Ile Pro Val Ala Ser Thr Leu Met Gly Leu
305 310 315 320

Gly Ser Tyr Pro Cys Asn Asp Glu Leu Ser Leu Gln Met Leu Gly Met
325 330 335

His Gly Thr Val Tyr Ala Asn Tyr Ala Val Glu His Ser Asp Leu Leu
340 345 350

Leu Ala Phe Gly Val Arg Phe Asp Asp Arg Val Thr Gly Lys Leu Glu
355 360 365

Ala Phe Ala Ser Arg Ala Lys Ile Val His Ile Asp Ile Asp Ser Ala
370 375 380

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Glu Ile Gly Lys Asn Lys Thr Pro His Val Ser Val Cys Gly Asp Val
385                 390                 395                 400

Lys Leu Ala Leu Gln Gly Met Asn Lys Val Leu Glu Asn Arg Ala Glu
                405                 410                 415

Glu Leu Lys Leu Asp Phe Gly Val Trp Arg Ser Glu Leu Ser Glu Gln
            420                 425                 430

Lys Gln Lys Phe Pro Leu Ser Phe Lys Thr Phe Gly Glu Ala Ile Pro
        435                 440                 445

Pro Gln Tyr Ala Ile Gln Ile Leu Asp Glu Leu Thr Glu Gly Lys Ala
    450                 455                 460

Ile Ile Ser Thr Gly Val Gly Gln Arg Gln Met Trp Ala Ala Gln Phe
465                 470                 475                 480

Tyr Lys Tyr Arg Lys Pro Arg Gln Trp Leu Ser Ser Ser Gly Leu Gly
                485                 490                 495

Ala Met Gly Phe Gly Leu Pro Ala Ala Ile Gly Ala Ser Val Ala Asn
            500                 505                 510

Pro Asp Ala Ile Val Val Asp Ile Asp Gly Asp Gly Ser Phe Ile Met
        515                 520                 525

Asn Val Gln Glu Leu Ala Thr Ile Arg Val Glu Asn Leu Pro Val Lys
    530                 535                 540

Ile Leu Leu Leu Asn Asn Gln His Leu Gly Met Val Met Gln Trp Glu
545                 550                 555                 560

Asp Arg Phe Tyr Lys Ala Asn Arg Ala His Thr Tyr Leu Gly Asp Pro
                565                 570                 575

Ala Arg Glu Asn Glu Ile Phe Pro Asn Met Leu Gln Phe Ala Gly Ala
            580                 585                 590

Cys Gly Ile Pro Ala Ala Arg Val Thr Lys Lys Glu Glu Leu Arg Glu
        595                 600                 605

Ala Ile Gln Thr Met Leu Asp Thr Pro Gly Pro Tyr Leu Leu Asp Val
    610                 615                 620

Ile Cys Pro His Gln Glu His Val Leu Pro Met Ile Pro Asn Gly Gly
625                 630                 635                 640

Thr Phe Lys Asp Val Ile Thr Glu Gly Asp Gly Arg Thr Lys Tyr
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<213> Brassica napus

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Leu Thr Pro Gln Lys Pro Ser Ser Arg Leu His Arg Pro Leu Ala Ile
        35                  40                  45

Ser Ala Val Leu Asn Ser Pro Val Asn Val Ala Pro Glu Lys Thr Asp
    50                  55                  60

Lys Ile Lys Thr Phe Ile Ser Arg Tyr Ala Pro Asp Glu Pro Arg Lys
65                  70                  75                  80

Gly Ala Asp Ile Leu Val Glu Ala Leu Glu Arg Gln Gly Val Glu Thr
                85                  90                  95

Val Phe Ala Tyr Pro Gly Gly Ala Ser Met Glu Ile His Gln Ala Leu
            100                 105                 110

Thr Arg Ser Ser Thr Ile Arg Asn Val Leu Pro Arg His Glu Gln Gly
        115                 120                 125

Gly Val Phe Ala Ala Glu Gly Tyr Ala Arg Ser Ser Gly Lys Pro Gly
    130                 135                 140

Ile Cys Ile Ala Thr Ser Gly Pro Gly Ala Thr Asn Leu Val Ser Gly
145                 150                 155                 160

Leu Ala Asp Ala Met Leu Asp Ser Val Pro Leu Val Ala Ile Thr Gly
                165                 170                 175

Gln Val Pro Arg Arg Met Ile Gly Thr Asp Ala Phe Gln Glu Thr Pro
            180                 185                 190

Ile Val Glu Val Thr Arg Ser Ile Thr Lys His Asn Tyr Leu Val Met
        195                 200                 205

Asp Val Asp Asp Ile Pro Arg Ile Val Gln Glu Ala Phe Phe Leu Ala
    210                 215                 220
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Thr Ser Gly Arg Pro Gly Pro Val Leu Val Asp Val Pro Lys Asp Ile
225                 230                 235                 240

Gln Gln Gln Leu Ala Ile Pro Asn Trp Asp Gln Pro Met Arg Leu Pro
                245                 250                 255

Gly Tyr Met Ser Arg Leu Pro Gln Pro Pro Glu Val Ser Gln Leu Gly
            260                 265                 270

Gln Ile Val Arg Leu Ile Ser Glu Ser Lys Arg Pro Val Leu Tyr Val
        275                 280                 285

Gly Gly Gly Ser Leu Asn Ser Ser Glu Glu Leu Gly Arg Phe Val Glu
    290                 295                 300

Leu Thr Gly Ile Pro Val Ala Ser Thr Leu Met Gly Leu Gly Ser Tyr
305                 310                 315                 320

Pro Cys Asn Asp Glu Leu Ser Leu Gln Met Leu Gly Met His Gly Thr
                325                 330                 335

Val Tyr Ala Asn Tyr Ala Val Glu His Ser Asp Leu Leu Leu Ala Phe
            340                 345                 350

Gly Val Arg Phe Asp Asp Arg Val Thr Gly Lys Leu Glu Ala Phe Ala
        355                 360                 365

Ser Arg Ala Lys Ile Val His Ile Asp Ile Asp Ser Ala Glu Ile Gly
    370                 375                 380

Lys Asn Lys Thr Pro His Val Ser Val Cys Gly Asp Val Lys Leu Ala
385                 390                 395                 400

Leu Gln Gly Met Asn Lys Val Leu Glu Asn Arg Ala Glu Glu Leu Lys
                405                 410                 415

Leu Asp Phe Gly Val Trp Arg Ser Glu Leu Ser Glu Gln Lys Gln Lys
            420                 425                 430

Phe Pro Leu Ser Phe Lys Thr Phe Gly Glu Ala Ile Pro Pro Gln Tyr
        435                 440                 445

Ala Ile Gln Val Leu Asp Glu Leu Thr Gln Gly Lys Ala Ile Ile Xaa
    450                 455                 460

Thr Gly Val Gly Gln His Gln Met Trp Ala Ala Gln Phe Tyr Lys Tyr
465                 470                 475                 480

Arg Lys Pro Arg Gln Trp Leu Ser Ser Ser Gly Leu Gly Ala Met Gly
                485                 490                 495

Phe Gly Leu Pro Ala Ala Ile Gly Ala Ser Val Ala Asn Pro Asp Ala
            500                 505                 510
```

Ile Val Val Asp Ile Asp Gly Asp Gly Ser Phe Ile Met Asn Val Gln
515 520 525

Glu Leu Ala Thr Ile Arg Val Glu Asn Leu Pro Val Lys Ile Leu Leu
530 535 540

Leu Asn Asn Gln His Leu Gly Met Val Met Gln Leu Glu Asp Arg Phe
545 550 555 560

Tyr Lys Ala Asn Arg Ala His Thr Tyr Leu Gly Asp Pro Ala Arg Glu
565 570 575

Asn Glu Ile Phe Pro Asn Met Leu Gln Phe Ala Gly Ala Cys Gly Ile
580 585 590

Pro Ala Ala Arg Val Thr Lys Lys Glu Glu Leu Arg Glu Ala Ile Gln
595 600 605

Thr Met Leu Asp Thr Pro Gly Pro Tyr Leu Leu Asp Ala Ile Cys Pro
610 615 620

His Gln Glu His Val Leu Pro Met Ile Pro Ser Gly Gly Thr Phe Lys
625 630 635 640

Asp Val Ile Thr Glu Gly Asp Gly Arg Thr Lys Tyr
645 650

<210> 103
<211> 655
<212> PRT
<213> Brassica napus

<400> 103
Met Ala Ala Ala Thr Ser Ser Ser Pro Ile Ser Leu Thr Ala Lys Pro
1 5 10 15

Ser Ser Lys Ser Pro Leu Pro Ile Ser Arg Phe Ser Leu Pro Phe Ser
20 25 30

Leu Thr Pro Gln Lys Asp Ser Ser Arg Leu His Arg Pro Leu Ala Ile
35 40 45

Ser Ala Val Leu Asn Ser Pro Val Asn Val Ala Pro Pro Ser Pro Glu
50 55 60

Lys Thr Asp Lys Asn Lys Thr Phe Val Ser Arg Tyr Ala Pro Asp Glu
65 70 75 80

Pro Arg Lys Gly Ala Asp Ile Leu Val Glu Ala Leu Glu Arg Gln Gly
85 90 95

Val Glu Thr Val Phe Ala Tyr Pro Gly Gly Ala Ser Met Glu Ile His
100 105 110

Gln Ala Leu Thr Arg Ser Ser Thr Ile Arg Asn Val Leu Pro Arg His
115 120 125

Glu Gln Gly Gly Val Phe Ala Ala Glu Gly Tyr Ala Arg Ser Ser Gly
130 135 140

Lys Pro Gly Ile Cys Ile Ala Thr Ser Gly Pro Gly Ala Thr Asn Leu
145 150 155 160

Val Ser Gly Leu Ala Asp Ala Met Leu Asp Ser Val Pro Leu Val Ala
165 170 175

Ile Thr Gly Gln Val Pro Arg Arg Met Ile Gly Thr Asp Ala Phe Gln
180 185 190

Glu Thr Pro Ile Val Glu Val Thr Arg Ser Ile Thr Lys His Asn Tyr
195 200 205

Leu Val Met Asp Val Asp Asp Ile Pro Arg Ile Val Gln Glu Ala Phe
210 215 220

Phe Leu Ala Thr Ser Gly Arg Pro Gly Pro Val Leu Val Asp Val Pro
225 230 235 240

Lys Asp Ile Gln Gln Gln Leu Ala Ile Pro Asn Trp Asp Gln Pro Met
245 250 255

Arg Leu Pro Gly Tyr Met Ser Arg Leu Pro Gln Pro Pro Glu Val Ser
260 265 270

Gln Leu Gly Gln Ile Val Arg Leu Ile Ser Glu Ser Lys Arg Pro Val
275 280 285

Leu Tyr Val Gly Gly Gly Ser Leu Asn Ser Ser Glu Glu Leu Gly Arg
290 295 300

Phe Val Glu Leu Thr Gly Ile Pro Val Ala Ser Thr Leu Met Gly Leu
305 310 315 320

Gly Ser Tyr Pro Cys Asn Asp Glu Leu Ser Leu Gln Met Leu Gly Met
325 330 335

His Gly Thr Val Tyr Ala Asn Tyr Ala Val Glu His Ser Asp Leu Leu
340 345 350

Leu Ala Phe Gly Val Arg Phe Asp Asp Arg Val Thr Gly Lys Leu Glu
355 360 365

Ala Phe Ala Ser Arg Ala Lys Ile Val His Ile Asp Ile Asp Ser Ala
370 375 380

Glu Ile Gly Lys Asn Lys Thr Pro His Val Ser Val Cys Gly Asp Val
385 390 395 400

```
Lys Leu Ala Leu Gln Gly Met Asn Lys Val Leu Glu Asn Arg Ala Glu
                405                 410                 415

Glu Leu Lys Leu Asp Phe Gly Val Trp Arg Ser Glu Leu Ser Glu Gln
            420                 425                 430

Lys Gln Lys Phe Pro Leu Ser Phe Lys Thr Phe Gly Glu Ala Ile Pro
        435                 440                 445

Pro Gln Tyr Ala Ile Gln Ile Leu Asp Glu Leu Thr Glu Gly Lys Ala
    450                 455                 460

Ile Ile Ser Thr Gly Val Gly Gln His Gln Met Trp Ala Ala Gln Phe
465                 470                 475                 480

Tyr Lys Tyr Arg Lys Pro Arg Gln Trp Leu Ser Ser Ser Gly Leu Gly
                485                 490                 495

Ala Met Gly Phe Gly Leu Pro Ala Ala Ile Gly Ala Ser Val Ala Asn
            500                 505                 510

Pro Asp Ala Ile Val Val Asp Ile Asp Gly Asp Gly Ser Phe Ile Met
        515                 520                 525

Asn Val Gln Glu Leu Ala Thr Ile Arg Val Glu Asn Leu Pro Val Lys
    530                 535                 540

Ile Leu Leu Leu Asn Asn Gln His Leu Gly Met Val Met Gln Trp Glu
545                 550                 555                 560

Asp Arg Phe Tyr Lys Ala Asn Arg Ala His Thr Tyr Leu Gly Asp Pro
                565                 570                 575

Ala Arg Glu Asn Glu Ile Phe Pro Asn Met Leu Gln Phe Ala Gly Ala
            580                 585                 590

Cys Gly Ile Pro Ala Ala Arg Val Thr Lys Lys Glu Glu Leu Arg Glu
        595                 600                 605

Ala Ile Gln Thr Met Leu Asp Thr Pro Gly Pro Tyr Leu Leu Asp Val
    610                 615                 620

Ile Cys Pro His Gln Glu His Val Leu Pro Met Ile Pro Ser Gly Gly
625                 630                 635                 640

Thr Phe Lys Asp Val Ile Thr Glu Gly Asp Gly Arg Thr Lys Tyr
                645                 650                 655
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<210> 104
<211> 652
<212> PRT
<213> Brassica napus

<400> 104

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Met Ala Ala Ala Thr Ser Pro Ser Pro Ile Ser Leu Thr Ala Lys Pro
1               5                   10                  15

Ser Ser Lys Ser Pro Leu Pro Ile Ser Arg Phe Ser Leu Pro Phe Ser
            20                  25                  30

Leu Thr Pro Gln Lys Pro Ser Ser Arg Leu His Arg Pro Leu Ala Ile
        35                  40                  45

Ser Ala Val Leu Asn Ser Pro Val Asn Val Ala Pro Glu Lys Thr Asp
    50                  55                  60

Lys Ile Lys Thr Phe Ile Ser Arg Tyr Ala Pro Asp Glu Pro Arg Lys
65                  70                  75                  80

Gly Ala Asp Ile Leu Val Glu Ala Leu Glu Arg Gln Gly Val Glu Thr
                85                  90                  95

Val Phe Ala Tyr Pro Gly Gly Ala Ser Met Glu Ile His Gln Ala Leu
            100                 105                 110

Thr Arg Ser Ser Thr Ile Arg Asn Val Leu Pro Arg His Glu Gln Gly
        115                 120                 125

Gly Val Phe Ala Ala Glu Gly Tyr Ala Arg Ser Ser Gly Lys Pro Gly
    130                 135                 140

Ile Cys Ile Ala Thr Ser Gly Pro Gly Ala Thr Asn Leu Val Ser Gly
145                 150                 155                 160

Leu Ala Asp Ala Met Leu Asp Ser Val Pro Leu Val Ala Ile Thr Gly
                165                 170                 175

Gln Val Pro Arg Arg Met Ile Gly Thr Asp Ala Phe Gln Glu Thr Pro
            180                 185                 190

Ile Val Glu Val Thr Arg Ser Ile Thr Lys His Asn Tyr Leu Val Met
        195                 200                 205

Asp Val Asp Asp Ile Pro Arg Ile Val Gln Glu Ala Phe Phe Leu Ala
    210                 215                 220

Thr Ser Gly Arg Pro Gly Pro Val Leu Val Asp Val Pro Lys Asp Ile
225                 230                 235                 240

Gln Gln Gln Leu Ala Ile Pro Asn Trp Asp Gln Pro Met Arg Leu Pro
                245                 250                 255

Gly Tyr Met Ser Arg Leu Pro Gln Pro Pro Glu Val Ser Gln Leu Gly
            260                 265                 270
```

```
Gln Ile Val Arg Leu Ile Ser Glu Ser Lys Arg Pro Val Leu Tyr Val
        275                 280                 285

Gly Gly Gly Ser Leu Asn Ser Ser Glu Glu Leu Gly Arg Phe Val Glu
    290                 295                 300

Leu Thr Gly Ile Pro Val Ala Ser Thr Leu Met Gly Leu Gly Ser Tyr
305                 310                 315                 320

Pro Cys Asn Asp Glu Leu Ser Leu Gln Met Leu Gly Met His Gly Thr
                325                 330                 335

Val Tyr Ala Asn Tyr Ala Val Glu His Ser Asp Leu Leu Leu Ala Phe
            340                 345                 350

Gly Val Arg Phe Asp Asp Arg Val Thr Gly Lys Leu Glu Ala Phe Ala
        355                 360                 365

Ser Arg Ala Lys Ile Val His Ile Asp Ile Asp Ser Ala Glu Ile Gly
    370                 375                 380

Lys Asn Lys Thr Pro His Val Ser Val Cys Gly Asp Val Lys Leu Ala
385                 390                 395                 400

Leu Gln Gly Met Asn Lys Val Leu Glu Asn Arg Ala Glu Glu Leu Lys
                405                 410                 415

Leu Asp Phe Gly Val Trp Arg Ser Glu Leu Ser Glu Gln Lys Gln Lys
            420                 425                 430

Phe Pro Leu Ser Phe Lys Thr Phe Gly Glu Ala Ile Pro Pro Gln Tyr
        435                 440                 445

Ala Ile Gln Val Leu Asp Glu Leu Thr Gln Gly Lys Ala Ile Ile Ser
    450                 455                 460

Thr Gly Val Gly Gln His Gln Met Trp Ala Ala Gln Phe Tyr Lys Tyr
465                 470                 475                 480

Arg Lys Pro Arg Gln Trp Leu Ser Ser Ser Gly Leu Gly Ala Met Gly
                485                 490                 495

Phe Gly Leu Pro Ala Ala Ile Gly Ala Ser Val Ala Asn Pro Asp Ala
            500                 505                 510

Ile Val Val Asp Ile Asp Gly Asp Gly Ser Phe Ile Met Asn Val Gln
        515                 520                 525

Glu Leu Ala Thr Ile Arg Val Glu Asn Leu Pro Val Lys Ile Leu Leu
    530                 535                 540

Leu Asn Asn Gln His Leu Gly Met Val Met Gln Trp Glu Asp Arg Phe
545                 550                 555                 560
```

```
Tyr Lys Ala Asn Arg Ala His Thr Tyr Leu Gly Asp Pro Ala Arg Glu
                565                 570                 575

Asn Glu Ile Phe Pro Asn Met Leu Gln Phe Ala Gly Ala Cys Gly Ile
            580                 585                 590

Pro Ala Ala Arg Val Thr Lys Lys Glu Glu Leu Arg Glu Ala Ile Gln
        595                 600                 605

Thr Met Leu Asp Thr Pro Gly Pro Tyr Leu Leu Asp Val Ile Cys Pro
    610                 615                 620

His Gln Glu His Val Leu Pro Met Ile Pro Ser Gly Gly Thr Phe Glu
625                 630                 635                 640

Asp Val Ile Thr Glu Gly Asp Gly Arg Thr Lys Tyr
                645                 650


<210> 105
<211> 10
<212> PRT
<213> Brassica napus

<400> 105
Ile Pro Ser Gly Gly Thr Phe Lys Asp Val
  1               5                  10


<210> 106
<211> 30
<212> DNA
<213> Brassica napus

<400> 106
atcccaagtg gtggcacttt caaagatgta                                   30


<210> 107
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      primer

<400> 107
catctttgaa agtgccacca c                                            21
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<210> 108
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 108
Ile Pro Asn Gly Gly Thr Phe Lys Asp Val
1 5 10

<210> 109
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
primer

<400> 109
atcccaaatg gtggcacttt caaagatgta 30

<210> 110
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
primer

<400> 110
catctttgaa agtgccacca t 21

<210> 111
<211> 10
<212> PRT
<213> Brassica napus

<400> 111
Met Gln Trp Glu Asp Arg Phe Tyr Lys Ala
1 5 10

<210> 112
<211> 30
<212> DNA
<213> Brassica napus

<400> 112
atgcaatggg aagatcggtt ctacaaagct 30

<210> 113
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
primer

<400> 113
ctttgtagaa ccgatcttcc c 21

<210> 114
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 114
Met Gln Leu Glu Asp Arg Phe Tyr Lys Ala
1 5 10

<210> 115
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
primer

<400> 115
atgcaattgg aagatcggtt ctacaaagct 30

<210> 116
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
primer

<400> 116
ctttgtagaa ccgatcttcc a 21